1. PURPOSE

To define standards and responsibilities for mouse housing and mouse breeding cages and to assign responsibility for action when mouse breeding cages are overcrowded.

2. POLICY

2.1. Investigators are responsible for:
   
   2.1.1. Coordinating with ACVS for space allocation of their mouse breeding colonies.
   
   2.1.2. Managing their own breeding colonies unless arrangements for technical support have been made with ACVS.
   
   2.1.3. Designating a Colony Manager (someone who has received specific training on managing mouse breeding colonies, and who will be the primary contact person for the lab). The PI may designate more than one Colony Manager, but it is preferable that this number be minimized to facilitate communication between the ACVS and the mouse users.

   2.2. The designated Colony Manager (research staff or ACVS staff) is responsible for separating animals according to allowed cage space.

   2.3. Both the ACVS staff and investigators managing their own mouse breeding colonies must abide by procedures as outlined in this document.

   2.4. Any recurring problems with mouse breeding colony management will be brought to the attention of the ACC.

3. PROCEDURE

3.1. BREEDING SCHEMES

   3.1.1. Two different breeding schemes are acceptable. In either case, the designated Colony Manager (research staff or ACVS staff) is responsible for carefully monitoring pregnancies.

   3.1.2. When setting up breeding schemes females should be added to the male cage.

   3.1.3. Monogamous pairs

      3.1.3.1. Postpartum estrus occurs within 24 hours of parturition; thus if male is left in the cage, the female is likely to become pregnant again while lactating and nursing new the litter.

      3.1.3.2. One male (only one male per cage is allowed) and one female are housed together for mating.

      3.1.3.3. Nesting material is provided in the cage.

      3.1.3.4. The mice are not separated when the female becomes pregnant or delivers the pups. This model takes advantage of postpartum estrus and allows the female to become pregnant and nurse at the same time.
3.1.3.5. Litters are born approximately 21 days apart. The 3 week old litter must be weaned prior to the birth of the new litter.

3.1.4. *Harem mating*

3.1.4.6. This method houses two (2) or maximum three (3) females in a cage with one male (only one male per cage is allowed).

3.1.4.7. During routine health/breeding checks, each noticeably pregnant female is removed and placed in her own individual cage. When the pregnant female is separated from the harem cage, she is given nesting material in her delivery cage to make a nest for her pups.

3.1.4.8. Female delivers her pups and nurses them for 21 (or up to 28 days, with ACVS approval, following the procedure below).

3.1.4.9. Only one nursing female and litter is allowed per cage. After the pups are weaned, the female may be returned to a harem cage.

3.1.4.10. Alternatively, when one or more females are noticeably pregnant, the male is removed and housed singly in a fresh cage. The singly housed male is given environmental enrichment according to ACVS SOP. Females deliver their pups and nurse them for 21 (or up to 28 days, with ACVS approval, following the procedure below). Housing two nursing females with litters in one cage requires prior ACVS approval. After the pups are weaned, the female(s) may be returned to a harem cage.

3.1.5. The Designated Colony Manager has primary responsibility for checking for pregnancy and birth and for recording these events on the cage card(s). When the litter is born, the cage is flagged with a New Litter card and the Date of Birth (DOB) and projected weaning date is documented. However, if ACVS staff finds new litters when checking and changing cages, they will place a new litter card and the DOB.

3.1.6. After pups are born, the cage is left undisturbed for at least three (3) days except for replenishing of food and water as needed.

3.1.6.11. In case the bedding gets very dirty or wet and the cage must be changed sooner, the following procedure will be followed. The female is transferred first, and then the litter plus nesting material is scooped up altogether with a gloved hand and transferred to the new cage. The same procedure is followed until the pups start moving around the entire cage.

3.2. **REQUIRED DOCUMENTATION AND CAGE CARDS**

3.2.1. Investigators may use ACVS cage cards as described below. Every animal must be identified on a cage card, either as a single card or a group of animals on one card.

3.2.2. Cage card (INSERT CAGE CARD, ADD ARROWS)

3.2.2.12. Required information:

- Principal Investigator
- AUP
- Student
- Strain and Genotype
- Supplier (e.g. Charles River or “in house” for animals bred in house)
3.2.3. Breeding card

3.2.3.13. Required information:

- AUP
- Principal Investigator
- Student/Colony Manager
- Female: ID, DOB, Strain
- Male: ID, DOB, Strain
- Date Female(s) and Male paired and separated
- Pups: DOB, # pups born, # weaned, DOW

3.2.4. New litter card (so that it is visible, this card is placed vertically behind the cage card)

- Noted if female noticeably pregnant
- Date of Birth (DOB)
• Weaning date of new litter

3.2.5. Colony Tracking sheet (see Appendix 1 for tracking sheet)

3.2.5.14. Colony Tracking sheets are maintained for each strain in a colony and can be found in the colony holding room.

3.2.5.15. Records must be maintained to determine performance of individual mice or strains of mice. The breeding indices outlined below can be useful in determining when individual mice are in need of being replaced as breeding stock or if problems have arisen in a colony.

3.2.5.16. Tracking sheets are submitted, by the Colony Managers each month to the University Veterinarian and subsequently the Animal Care Committee.

3.3. WEANING PUPS

3.3.1. Age of pups at weaning

3.3.1.17. Weaning age for mouse pups is routinely 21 days of age. In the case of some inbred, genetically modified or mutant strains, it may be advantageous to allow the pups to remain with the female for 28 days.

3.3.1.18. To extend nursing time past the 21 day standard, ACVS must be notified by placing a note on the New Litter card. The Colony Manager must communicate via email to the University Veterinarian or ACVStech@carleton.ca when all pups in a given breeding line will be routinely weaned at 28 days.

3.3.1.19. Allowing a 3 week old litter to stay in the cage with a lactating female who also has a newborn litter is not permitted.

3.3.2. Monogamous pairs
3.3.2.20. Assuming the lactating mother is pregnant, pups are weaned at 21 days of age, just before the new litter is born. This will prevent trampling of newborn pups by the weanling pups, and prevent the cage from being overcrowded.

3.3.3. *Harem mated females*

3.3.3.21. If a singly housed lactating female is alone in a cage with her litter, weaning is less urgent than with monogamous pairs. However, mouse pups are routinely weaned at 21 days of age unless an exception has been approved by the ACVS.

3.3.3.22. If two females raise their pups together in one cage, a procedure which must be preapproved by ACVS, attention must be paid as the pups grow older so that the cage does not become overcrowded. Each female with litter must be singly housed no later than two weeks after birth of pups.

3.3.4. Separation of sexes at weaning

3.3.4.23. Male and female pups are separated at weaning; mice of each sex being placed in a separate cage.

3.3.4.24. If a litter contains only one pup of a given sex, provisions must be made to house this pup with others of the same sex. Newly weaned pups must not be housed singly.

3.3.4.25. Possible housing options include:

3.3.4.26. A single female pup may remain with the mother.

3.3.4.27. A single male pup may be placed with other male pups from a different litter of the same age.

3.3.4.28. If the parents are a monogamous pair, a single male pup may be housed with the father, both being separated out into a new cage.

3.3.5. It is highly recommended that sexing of the pups be verified one week later.

3.3.6. Feeding of weaned pups

3.3.6.29. At the time of weaning, a small amount of chow must be provided on the cage floor for the next seven (7) days.

3.4. **HOW MANY MICE ARE ALLOWED PER CAGE**

3.4.1. All types mouse cages including shoe box and IVC

3.4.2. When mice are weaned, they are weaned to adult specifications to avoid needing to separate them and rehouse them in 2-3 weeks.

3.4.3. Four adult (4) female mice or four (4) male mice OR one lactating female with nursing litter are allowed per cage.

3.4.4. One adult male and one adult female with or without nursing litter (monogamous pair).

3.4.5. Occasionally when females have small litters or do not lactate well it may be beneficial to house two lactating females together in one cage so they can raise their litters cooperatively. This must be preapproved by ACVS, noted on the cage card and communicated via email to ACVS.

3.4.6. One adult male and two (2) or maximum three (3) adult females without litters (harem cage).
3.5. **DEFINING RESPONSIBILITY FOR SEPARATING AND WEANING MICE**

3.5.1. The designated Colony Manager (research staff or ACVS staff) is responsible for cage card documentation and for separating and weaning according to above guidelines.

3.6. Nonbreeding experimental mice are separated by PI or research staff unless technical support has been arranged with ACVS in advance.

3.7. **ACVS ACTIONS WHEN PI-MANAGED CAGES HAVE BECOME OVERCROWDED (O/C)**

3.7.1. The ACVS Staff checks for O/C and pregnancy when performing daily health checks and when changing cages. Any cages that are overcrowded according to standards defined above are marked with a Problem Notification – O/C card, dated and initialed.

3.7.2. When overcrowding is noted, the responsible individual, the designated Colony Manager, is contacted via email and given 48 hours to correct the problem, depending on the severity, if not performed ACVS will separate the mice.

3.7.3. Males that are fighting or have fight wounds. Separate promptly.

3.7.4. When a harem housed mouse is noticeably pregnant (usually 14 days gestation), she must be separated from the male and other females within 48 hours. However, if female seems to be about to give birth, she is promptly separated.

3.7.5. When two litters, one newborn and one previous litter, are in one cage, separation is performed as soon as possible. In such a case, the ACVS Staff separates the older pups into separate cages and gives a few food pellets on the cage floor.

3.7.5.30. Female and newborn pups are left in the breeding cage. Cage is marked with a Problem Notification – O/C card, and any other information necessary to identify the mice, dated and initialed.

3.7.6. Any time a cage is significantly O/C and the welfare of the animals is at stake (Emergency O/C), the animals are promptly separated into acceptable group sizes.

3.7.7. When the overcrowding is corrected the O/C card is removed.
### 4. APPENDIX 1

<table>
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<th>Date</th>
<th>Total Pups Born</th>
<th>Pups found dead or killed</th>
<th># pups euth'd before weaning</th>
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