



Division of Research

SUBJECT: Rodent Breeding Colony Management	Effective Date: 5/31/2024	Policy Number: 10.4.20
	Supersedes: N/A	Page of 1 6
	Responsible Authorities: Principal Investigator Vice President, Research Institutional Animal Care and Use Committee Associate Vice President for Research, Research Integrity Assistant Vice President for Research, Comparative Medicine	

- I. Background
 The Public Health Service (PHS) policy and the “Guide for the Care and Use of Laboratory Animals” (Guide) require the Institutional Animal Care and Use Committee (IACUC) and the Attending Veterinarian to provide oversight of all experimental procedures including breeding of research animals and guarantee the welfare of the animals used in biomedical research. As stated in the Guide, carefully designed breeding strategies and associated procedures can minimize the generation of unwanted animals. This also addresses genetic drift of genetically manipulated animals and therefore contributes to the 3R’s due to reduction of the overall number of animals and reproducibility of specific animal models.

- II. Purpose
 The purpose of this document is to provide guidelines and establish parameters for proper rodent breeding activities including breeding schemes, timely retirement of breeders, appropriate genotyping and weaning of their offspring, regulated space requirements for housing, possible cross fostering, and adequate recordkeeping associated with these activities. This document applies to all researchers and to a limited extend to veterinary care staff involved in breeding colony management. Exceptions to this policy require prior IACUC approval.

- III. General Statement
 Rodent breeding colonies are maintained to produce experimental animals and preserve irreplaceable lines/strains. Researchers must justify the establishment and continuation of existing rodent breeding colonies since many of the specific stocks, strains, and lines of mice and rats are commercially available. Investigators must avoid unnecessary production of animals and abide to housing space requirements described in the Guide. Aptly executed activities associated with breeding colony management protect the welfare of animals and provide physiologically stable subjects for animal research models.

IV. Policy

- A. Each procedure with animals including breeding must be described in the IACUC protocol and approved by the IACUC before execution.
- B. The Principal Investigator (PI) must justify the need for the establishment of a new or continuation of an existing breeding colony in the IACUC protocol. This can include but is not limited to:
 1. Breeding genetic lines that are not commercially available.
 2. Intercrossing genetically modified animals for an experiment that requires specific genotypes.
 3. Animals needed at a specific age or weight requirement that cannot be fulfilled by a commercial source.
 4. Cost alone is not a valid justification.
- C. During times when experiments do not require continued production of animals but will recur in the near future breeding might be continued at a lower scale to maintain the particular stock, strain, or line. The decision to continue breeding needs to be reviewed regularly to avoid unnecessary euthanasia of animals without a scientific need.
- D. If a particular breeding colony has not been producing animals for experiments for more than 3 years the colony it is strongly recommended the cryo-preserve the strain/line and discontinue breeding.
- E. Researchers must track the numbers of animals to prevent exceeding approved animal numbers in a particular protocol, which is considered a non-compliance incident.
- F. Numbers of animals produced in a breeding colony must be kept to the minimum number required to meet scientific goals. The IACUC must be presented with sufficient information to verify that the opportunity for excessive animal production and/or use is minimized. This information should be based on the number of animals needed for experimental purposes and strain/line maintenance.
- G. PIs are responsible for overseeing all aspects of their research projects including breeding colony management and recordkeeping even when outsourced to Comparative Medicine (CM) staff. This includes the numbers of animals produced since CM veterinary staff does not have the information for avoidance of overproduction of offspring. However, whenever vet staff becomes aware of excessive production of pups they will advise the PI accordingly.
- H. Cage density management must be based on the Guide's space recommendations, which is two adults and one litter as maximum number of animals per cage. To avoid overcrowding weaning must be timely.
- I. Pups are to be weaned between 19 and 21 days of age. Exceptions to weaning age due to e.g., small pups must be either prescribed by the veterinary staff for a specific litter or requests for regularly occurring concerns in a specific strain/line described to, reviewed, and approved by the IACUC to allow extension of weaning time for that strain/line in general.
- J. Breeding pairs can be setup as monogamous pair or as harem breeders. If harem breeding is chosen pregnant females must be separated into individual maternity cages to avoid overcrowding once the litter(s) is delivered. The presence of two or more litters in a single cage is not permitted unless specifically justified and approved in the IACUC protocol.
- K. Breeding males must be singly housed after being separated from the female(s) due to their aggressive nature after breeding avoiding harm to another male cage mate.
- L. Female and male breeder might need to be single housed when there is no immediate need for a new litter or if females are separated for imminent parturition. The time of single housing should be minimized whenever possible, and a single housing sticker

completed with reason and start date for/of single housing must be affixed to the cage card.

- M. Fertility of rodents is highest during the first 6 months of their lives starting not earlier than 6 weeks of age and no later than 12 weeks of age to being productive.
- N. The PI or the veterinary staff member if breeding colony management is sourced to CM are responsible for monitoring pregnant females, litters, and pups once weaned.
- O. Recordkeeping and colony management practices must demonstrate efforts to utilize animals in ways that conserve genetic traits and are not wasteful. Regular genetic testing is recommended. Backcrossing genetically manipulated strains/lines to a male of the background strain is recommended every five (5) generations to avoid excessive genetic drift.
- P. If a new strain/line has been created for a specific experiment, the research personnel must monitor for adverse phenotypes that could affect the animals' well-being. If found that must be reported to the IACUC and the protocol amended to describe the abnormal/unexpected phenotype and a plan to manage the animals accordingly.

V. Definitions

- A. Monogamous or pair breeding: Breeding of one (1) adult male and one (1) adult female.
- B. Harem breeding: Breeding one (1) adult male with two (2) to three (3) adult females.
- C. Post partum estrus: Female rodents undergo a fertile estrus 14-24 hours following parturition. If breed as monogamous pair and the male is not removed from the cage the likelihood of the female conceiving is very high. This increases breeding productivity but also increases the likelihood of overcrowding due to a second litter being born while the first litter is not weaned. This requires very tight oversight.
- D. Co-parenting of two females: Two (2) females in a cage rearing a litter. This might increase survival of pups in low production strains but is an exception and needs approval by the IACUC.
- E. Pup: Neonatal rodent up to 21 days.
- F. Litter: Pups from the same litter and pregnancy.
- G. Weaning: The act of separating pups from the dam, usually 21 days.
- H. Overcrowding: More animals in a cage than allowed by the Guide, e.g., three adults and a litter, or two females and two litters per cage.

VI. Accountability

The Principal Investigator (PI) will be responsible for:

- Justify the need for the establishment of a new or continuation of an existing breeding colony and describe procedures including number justification and monitoring of the breeding colony in an IACUC proposal.
- Oversee all staff and students involved in breeding, provide appropriate training beyond the centralized training required by the IACUC, assure abidance to this policy.
- Plan colony management and assure that only as many offspring are approved needed for the proposed experiments, for successful continuation of strains/lines, and sharing of breeder animals with colleagues. Avoid unnecessary euthanasia.
- Assure timely weaning of litters avoiding overcrowding and potential loss of a second litter/smaller pups.
- Regular backcrossing and verification of genetic makeup of the strain.

The IACUC will be responsible for:

- Ensuring that the need for a breeding colony has been established based on scientific evidence and/or animal welfare concerns.
- Reviewing number of pups to be produced based on scientific justification.
- Approving all procedures related to the breeding process.
- Ensuring mechanisms for tracking of animal numbers.
- Regularly evaluating the standards of care and welfare for the animals in the breeding colony consistent with current policies and regulations.

The Research Integrity office will be responsible for:

- Administrative support of the IACUC members to facilitate their regulatory function.
- Maintaining policy and assure regular review and update as necessary by the IACUC.
- Assure mandatory training attendance for breeding colony maintenance, keep relevant training records, and provide to the IACUC for review.

The Office of Comparative Medicine (CM) will be responsible for:

- Abide to this policy when performing breeding colony management for and based on the information provided by the research lab that requests the service.
- Provide support and basic training for all personnel involved in breeding colony management.

VII. Procedures

- A. Any animal breeding must be described in the protocol. To avoid excessive animal production of offspring needs to be based on number of animals needed for experimental and strain/line maintenance. The calculation needs to include estimation of the number of pups per litter, the number of usable animals per litter (e.g., genotype, sex), the number of litters per dam, and number of dams/sires.
- B. Mice have an estrus cycle of 5 days and gestation is 21 days.
- C. Sexual maturity in mice is achieved at 6-8 weeks of age. Breeders must not be paired before 6 weeks of age since this might be detrimental to the breeding performance of the female. The breeding life span in mice is usually up to one (1) year of age but commonly shorter in most genetically manipulated strains. Fecundity will decrease after 6-8 months of age showing in prolonged intervals between pregnancies, no pregnancy at all, and smaller litters. To avoid losing a strain/genotype replace breeders latest after 8 months.
- D. Monogamous pairs can be kept together unless a new litter soon after parturition is not desired. If post-partum estrus will be utilized the first litter must be weaned before the birth of the new litter, which requires close monitoring of the breeding cage. It is advisable to affix a green "watch for pups" card strip to the cage.
- E. Harem breeding requires to monitor the females for pregnancy, which can be detected around the end of the second trimester, i.e., 2 weeks. Visibly pregnant females must be separated prior to parturition to avoid overcrowding.
- F. Many genetically manipulated strain/lines are poor breeders or have poor maternal instincts, especially associated with the first litter. Therefore, cages with newborn litters should not be disturbed, i.e., removed from the IVC rack and/or animals handled. CM personnel will not change cages with litters younger than 4 days unless for necessary provision of food/water or excessively soiled cages.
- G. The research personnel are responsible for watching breeder cages for newborn litters and attaching the green card strip completed with birthdate and wean date to the cage.

CM personnel will do this as well if new litters are found during daily health checks but will not manipulate the cage for better view.

- H. If the dam is sick including lactation problems or dies, any effort should be made to find an appropriate foster dam. Ideally, the foster dam should be caring for a litter of approximate age of the litter to be fostered and should have the history of good maternal instincts or be a strain know to have good mothers. The total pup number should not exceed the maximum number of pups typical for the foster dam strain. If litters are large, some pups may need to be euthanized or the litter to be fostered may need to be divided among multiple foster dams. It is important to gently rub the foster pups with the bedding/nesting material of the foster dam cage or allow the foster dam to urinate on the new pups while holding the dam up over all the pups.
- I. The weaning age for pups is 21 days. Litters must be weaned at 21 days or if the weaning falls on a weekend up to 23 days. If an extended weaning is necessary for specific situations there are two options for request of extension by research personnel:
 - 1. If it affects one litter (e.g. small pups) the veterinary staff can extend the weaning for veterinary medical reasons for this particular group of pups.
 - 2. If it affects a particular strain/line an exemption can be requested via an amendment/IACUC protocol. The request must be justified, reviewed, and approved by the IACUC.
 - 3. A wean extension tag must be secured to the cage.
- J. At time of weaning usually tissue will be collected for genotyping if necessary and a form of identification applied. Please check the IACUC Policy 10.4.8.
- K. During weaning separate pups into cages based on sex. If a litter contains only one pup of a given sex the pup should be housed with others of the same sex, i.e., from a different litter and about same age. If the dam has not been bred again the pup can also be housed with the mom for a few more weeks.
- L. Weaned pups often require additional support such as a diet gel or wetted food pellet(s) in a disposable paper cup, which are available in the vivarium. In housing facilities where autowater is the source of water, newly weaned pups must receive a water bottle for the first four (4) weeks after weaning since small pups need time to learn the use of the water valve and might have difficulties to reach it. Those cages must be flagged with a yellow "new to autowater" card strip and the date of weaning written on the strip. Also, toggle the water valve to check proper function when placing the cage on the IVC rack.
- M. Any cage containing more than two adults (female/male pair or two females w/ second female not being pregnant) and one litter is considered overcrowded per Guide. To avoid this, separate pregnant females from harem breeding cages and wean pups timely. If not corrected within 48 hours of the cage becoming overcrowded, CM personnel will rectify the situation and the time and effort needed for this will be charged to the appropriate account of the PI.
- N. Recordkeeping is important for maintaining a breeding colony. The records should include as a minimum mating pairings (identification of dam and sire), generation, number of animals produced and disposition of animals. This must be available to the IACUC for their review during inspections.

VIII. Policy Renewal Date
5/31/2027

IX. References

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POLICY APPROVAL

Initiating Authority

Signature: _____

Date: _____

Name: Gregg B. Fields, Ph.D., Interim Vice President for Research

Executed signature pages are available in the Initiating Authority Office(s)