POLICY REGARDING THE BREEDING OF RODENTS

PURPOSE:

Maintaining a breeding colony in a research setting may lead to unique scientific and animal welfare concerns. This document serves to outline the process for establishing and maintaining a rodent breeding protocol at Liberty University.

POLICY:

While maintaining an active breeding protocol, the Principal Investigator must:

- Coordinate and ensure appropriate space is allocated for the maintenance of a breeding colony.
- Manage rodent colonies consistent with the procedures outlined in an approved IACUC protocol.
- Designate a colony manager (person who has received specific training or has experience managing rodent breeding colonies).
    - This person shall serve as the primary contact regarding any breeding protocol concerns.
- Maintain appropriate colony records. The records must include the following (at minimum):
    - Mating Pairings
    - Generations
    - Number of animals produced
    - Number of animals transferred to protocols (to ensure no excess animals are used)

While maintaining an active breeding protocol, the designated colony manager must:

- Separate animals according to approved cage space allocations (to avoid overcrowding)
- Maintain appropriate colony records
- Ensure adequate care is given to all animals

Problems with colony management or breeding must be reported to the IACUC immediately.

BREEDING PROCEDURES

The PI and/or colony manager is responsible for monitoring pregnancies within rodent colonies. Liberty University has established two acceptable breeding methods:

1. Monogamous Pairs (One Male + One Female)
    a. Only one male per cage for 12 days (allows 2 cycles for impregnation)
    b. Nesting material must be provided in the cage
    c. Once mice are separated, female must be monitored daily for weight gain and delivery.
    d. Litters must be weaned at 4 weeks
        i. Note: Litters are born approximately 21 days apart
    e. Post-partum estrus occurs within 24 hours of parturition.
        i. Males must be removed 12 days after pairing so that they will not be in the cage at this time.
2. Harem Mating (One Male + Two-Three Females)
    a. Only one male per cage for 12 days (allows 2 cycles for impregnation)

b. Pregnant females must be removed from their cages and placed in separate birthing cages with appropriate nesting material.
    i. Only one nursing litter is allowed per cage
    ii. Pups are nursed for 21 days and up to 28 days (with IACUC approval)
    iii. Once pups are weaned, the female may be placed back in the harem cage

ROUTINE HEALTH CHECKS

Routine health checks are required as part of an active breeding protocol. Health checks must be performed as follows:

1. On a daily basis, the PI and/or colony manager must check for pregnancies and births
2. Health checks must be documented
3. If new litters are born, cages must be flagged with new litter cards
    a. Include the date of birth
    b. Include the projected weaning date

AFTER-BIRTH PROCEDURES

The after-birth process requires that the following procedures be followed:

1. Cages must be left undisturbed for at least three days, except for the following:
    a. Food and water replenishment
    b. Overly soiled or wet bedding
        i. Females must be transferred before the litter
        ii. A small amount of the original bedding shall be transferred to the fresh cage to allow pups to familiarize themselves with the scent

WEANING

The weaning process requires that the following procedures be followed:

1. Age
    a. The weaning age for pups is 21 days. In certain circumstances, weaning may be extended to 28 days (modified/mutant strains).
    b. For cases in which weaning must extend to 28 days:
        i. Note on the cage card that the litter will be on “Extended Weaning” along with the expected wean date.
    c. It is not appropriate, under any circumstance, for a three-week-old litter to remain in a cage with a lactating female and her pups.
2. Separation of Sexes
    a. Male and female pups are separated at weaning. Mice of each sex shall be placed in separate cages.
    b. If a litter contains only one pup of a given sex, the pup must be housed with others of the same sex. Newly weaned pups must not, under any circumstance, be singly housed.
        i. A single female pup may remain with the mother.
        ii. A single male pup may be placed with other male pups from a different litter of the same age.
        iii. If the parents are a monogamous pair, a single male pup may be housed together with the father in a new cage.

iv. A single male pup may be housed with female siblings up to six weeks of age (adulthood).
    1. More than one male pup may not be housed with female siblings.

c. Sexing of the pups must be verified after one week to ensure appropriate separation has occurred.
    i. This is performed by determining anogenital distance. For females, this distance is 1/3 to 1/2 the distance of the male.

3. Feeding
    a. At the time of weaning, rodent feed must be placed on the cage floor to span seven subsequent days.

OVERCROWDING

Cages must be monitored daily for pregnancy and birth. Cages that are overcrowded must be dealt with immediately upon notification.

1. If overcrowding is noted, the PI and/or colony manager has 72 hours (including weekends and holidays) to address the issue. If the issue is not addressed within this time frame, the IACUC will be notified and may suspend the protocol.
2. Fighting or wounded rodents must be separated as quickly as possible.
3. When a harem housed female is noticeably pregnant, she shall be separated within 48 hours following the initial observation. If the female is about to give birth, immediately separate her from the harem.
4. If two litters are in the same cage, they must be immediately separated.
    a. Older pups must be placed into separate cages with food or gel packs on the cage floors.
    b. Female and newborn pups may be left in the breeding cages.
5. Keep accurate documentation of any updated cage numbers upon birth or separation.

IACUC OVERSIGHT AND REVIEW

The IACUC will carefully review breeding colony protocols to ensure proper colony management is in place. This will include an in depth review of breeding schemes, weaning ages, and methods for identification of individual animals. The IACUC requires that the number of unusable animals be minimized to the greatest extent possible. PIs are encouraged to work with the IACUC to ensure that unusable animals be made available to other researchers whenever possible. If a species or strain is commercially available, the production of animals on a breeding colony must be scientifically justified. Cost and convenience is not considered a valid justification for developing a breeding colony.

BREEDING LIMITATIONS AND JUSTIFICATIONS

Investigators wishing to establish a breeding protocol must submit an application to the IACUC prior to starting work. All requests to add a breeding protocol to an existing protocol require full IACUC review. Each investigator is limited to a total of six active breeding cages at any given time. Exceptions may be granted on a case-by-case basis by the IACUC. Investigators with transgenic strains may request up to six active breeding cages per strain at any given time. Exceptions may be granted on a case-by-case basis by the IACUC. If there is any question as to whether or not breeding is appropriate for the protocol, contact the IACUC for further clarification.