WVU IACUC Policy and Guidelines: Breeding and Weaning of Mice and Rats

Purpose
This document establishes the parameters for appropriate rodent (mouse and rat) breeding activities under the WVU animal care and use program. It applies to all research personnel who list breeding activities in mice or rats on an approved animal use protocol. Exceptions to the policy or guidelines require prior IACUC approval. Any recurring issues with rodent breeding colony management should be brought to the attention of the IACUC.

Background
The IACUC’s role for oversight includes ensuring that the need for a breeding colony has been established based on scientific or animal welfare concerns (cost alone is not a valid justification), that the procedures used in the breeding colony are evaluated and approved by the IACUC on a regular basis, that there is a mechanism for tracking animals, and that the standards of care and animal well-being for the animals in the breeding colony are consistent with current policies and regulations.

Policy
● Breeding of animals must be scientifically justified and described in an IACUC-approved animal use protocol.
● Only one adult male rodent is permitted in a breeding cage.
● Female rodents must be at least six weeks old before they are placed with a sexually mature adult male for breeding. The only exception to this is superovulation of 3-4 week old female mice for collection of zygotes or embryos. Superovulated females should not be allowed to go to term.
● Males should NOT be re-housed together after they have been separated for breeding purposes, or if they originate from different aged litters.

General Information
Optimal reproductive age span: mice 2-10 months; rats 2-15 months

Estrous cycle: 4-5 days
Gestation: mice 18-21 days; rats 21-23 days

Weaning age: ≥ 19 days old, usually 21 days, can be up to 28 days if approved by the IACUC (see below – Exceptions)

Postpartum estrus: a period within 24 hours after parturition when females are fertile and can conceive. After this period, they are not fertile until the pups’ weaning age (usually ~ 21 days)

Adult: defined as 6 weeks of age or older, based on the average age of sexual maturity

The Office of Laboratory Animal Resources (OLAR) veterinary staff can provide training and additional information on best practices for rodent breeding to research staff, upon request.
Guidelines
A. Colony Management

1. General Information
   a. Breeding cages should be regularly monitored to ensure the well-being of adults and neonates, appropriate cage environment and colony breeding performance. Changes should be made when necessary (i.e. separating mice, moving animals to a clean cage, retiring breeders, etc.).
   b. Animal usage records should be maintained to track offspring and their ultimate disposition. Suggested information would include sire/dam #, date and # born, date and # weaned and final disposition (i.e. euthanized, died, used in experiment, transferred to another protocol, or retained for breeding).
   c. Record keeping and colony management practices should demonstrate efforts to utilize animals in ways that conserve genetic traits and are not wasteful.
   d. Investigators maintaining breeding colonies exclusively to preserve a genetic line of rodents should consider other strategies such as cryopreservation of ova, sperm and/or embryos.
   e. When newborn pups are found, the date of birth must be recorded on the cage card, and a white [DO NOT CHANGE] cling placed on the cage, per OLAR’s Mouse or Rat Husbandry SOP.
      ➢ OLAR staff typically take care of this, but research staff should follow the same practice if they note pups.

2. Breeding Schemes – MICE
   a. Monogamous pair (1 male, 1 female) in a standard housing cage. Male is not necessarily separated when the female becomes pregnant or delivers the pups. Litters are born approximately 21 days apart. The three-week-old litter must be weaned prior to the birth of the new litter.
   b. Breeding trios (1 male, 2 females) in a standard housing cage. Pregnant females must be separated prior to parturition, and only 1 litter of pups with up to 2 adults may remain in the cage after pups are born.
   c. Harem breeding (1 male, 3-4 females) in a standard housing cage. Pregnant females must be separated prior to parturition, and only 1 litter of pups with up to 2 adults may remain in the cage after pups are born.

3. Breeding Schemes – RATS
   a. Any breeding schemes used must be described under the breeding section in an IACUC-approved protocol. Consult with OLAR staff to determine options based on type of cages used.

4. Overcrowded Cages
   a. A cage is considered overcrowded if a new litter is born before the older litter from the same female is weaned. If postpartum estrus is used, the first litter must be weaned by 20 days of age to prevent the presence of two litters in a cage.
b. **MICE**
   1. No more than 2 adults and 1 litter, regardless of litter size, are allowed in a standard housing cage.
   2. Maximum of 5 adult mice may be housed per standard mouse cage. If there are more than five young mice in a cage, the date of birth **must** be written on the cage card, to indicate they are not adults. If the age of the mice is unclear, they **must** be housed at a maximum of 5 per cage.

c. **RATS** – the number of rats allowed per cage will vary depending on the type of cages used, and the size/weight of the rats. Consult with OLAR staff to determine maximum housing density.

5. **Weaning**
   a. Litters should be weaned by postnatal day 21, or before the birth of a second litter, unless there is an approved exception in the IACUC protocol (see below – Exceptions).
   b. Weanling animals should be placed in a clean, standard housing cage with food and water and sorted by sex. Regardless of the caging system, a fresh water bottle should be provided to newly weaned rodents.
   c. Food and water **must** be accessible to weaned animals. It is helpful to provide a few feed pellets and/or moist feed in a petri dish on the floor for easy access at the time of weaning.
   d. If cages of newly weaned pups need additional observation, contact OLAR staff for recommendations on how to identify the cages.
   e. OLAR staff will wean the litter if research staff does not perform this by postnatal day 22, or when a second new litter is born. **There is a charge for this service.** Continued failure of the research staff to wean in a timely manner will be considered a non-compliance issue and is reportable to the IACUC.

B. **Exceptions**
   1. **Extended weaning date:**
      If a litter of normal weaning age is judged to be not ready to wean, veterinary staff should be consulted. Examples include small pup size, slow growth rate, and inability to access food or water. Delayed weaning up to seven days can be approved by veterinary staff. The male should be removed until weaning is complete to avoid impregnation of the female during postpartum estrus. Cages are typically placed on clinical call when extending the weaning period in consultation with veterinary staff.

   2. **Allomothering:**
      In instances where allomothering (“aunting”) by a second female is known (by presenting data to that effect) to increase survival of pups, harem breeding may extend to nursing, if justified and approved by the IACUC. Some specialized genetically modified lines may require more than 2 adults and 1 litter in the breeding cage to facilitate adequate production. Breeding records demonstrating poor breeding **must** first be reviewed with the facility veterinarian and scientific justification submitted to the IACUC for approval. Larger caging may be necessary to comply with housing density recommendations in the *Guide.*
3. **Requests for a perpetual exception** to extend weaning date **must** be submitted to the IACUC. If approved, OLAR should also be contacted regarding how to identify the weaning exception at the cage level.

Some reasons for extending weaning date include:
- a. Experimental design, e.g. studying psychological imprinting of pups
- b. Need to prevent loss of litters due to cannibalism
- c. Need to extend nurturing time in strains that fail to thrive

C. Counting Animals *(see IACUC Counting Animals Procedures)*

- OLAR staff typically take care of this, but research staff should follow the same procedures if they remove pups before they are counted by OLAR staff.

**References**

2. Breeding Strategies for Maintaining Colonies of Laboratory Mice, The Jackson Laboratory.