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**UC Davis
Institutional Animal Care and Use Committee (IACUC)**

Title: Rodent Breeding Colony Policy

I. Purpose:

This policy outlines requirements for **non-USDA** rodent breeding colonies based on Public Health Service Policy and the ILAR Guide for the Care and Use of Laboratory Animals (*Guide*).

II. Policy:

Animal care for rodent breeding colonies must meet or exceed the minimum requirements outlined below. Any deviation from these standards must be described in an animal care and use protocol and approved by the IACUC.

It is the responsibility of the Principal Investigator (PI), the Husbandry staff, and the Veterinary staff to ensure proper breeding techniques, weaning procedures, and cage density requirements are adhered to. The PI and their delegates are responsible for maintaining the breeding colony; however, if they fail to meet these minimum standards, the vivarium has the authority and responsibility to ensure campus standards are met per the procedures outlined below. Failure to meet these standards may result in loss of the privilege to maintain rodent breeding colonies. The facility, veterinary staff, or other campus entities providing breeding management services may charge the PI's account for labor related to breeding, weaning, and separating of mice and rats, or for corrective action taken on the PI's behalf to bring all aspects of the breeding program into compliance. For example, if the husbandry staff must separate multiple litters in a cage, the facility may charge labor rates for the time spent performing this function.

III. Procedure:

A. Breeding Schemes and Management:

The procedures listed below were written with mice in mind but pertain to both mice and rats unless stated otherwise.

Breeding management will be performed by facility staff, the PI, or a combination of the two as determined prior to colony development and outlined in an IACUC approved protocol.

Breeding cages for mice can be set up in pairs, trios, or harems. Only one male is allowed per breeding cage, and the total number of adult mice per cage may not exceed cage capacity.

In standard mouse caging (designed for 4 or 5 adult mice), two litters may not be present at the same time, unless specified in the facility SOP or approved IACUC protocol. Certain facilities may have cages designed for more than 5 adult mice, which can house up to two females with one litter each. For trio and harem breeding, pregnant females should be identified and separated into their own cages with appropriate nesting/enrichment materials prior to birth. This can be accomplished via palpation or visualization at E15 (15 days of gestation) or after mating when a plug is identified. If a female gives birth while in the trio or harem cage, the male and remaining females should be removed to a separate cage leaving the female with her litter undisturbed. When pregnant females from a trio or harem cage are separated, the male may remain with one of the females, be moved to another breeding cage, or housed separately based on the needs of the colony/protocol. The male mouse may remain or be removed based on the cage density established by the facility.

Standard rat cages are sized only for pair breeding, and the male should be removed prior to parturition to ensure adequate space for the female and her litter.

When breeding mice in pairs (one male and one female), the dam and sire may remain together throughout gestation and lactation. Breeding pairs often breed during post-partum estrus (immediately following parturition) thus pairs with litters near weaning age must be monitored closely for the arrival of a new litter. Ideally, the current litter should be weaned just prior to the birth of the new litter. However, if a new litter arrives early, the older litter must be weaned even if it is not yet 21 days old or the new litter humanely euthanized.

B. Cross Fostering:

If a dam fails to provide maternal care, has inadequate milk supply, must be humanely euthanized, or dies, the litter may be cross fostered to a surrogate mother. Some specialty or transgenic strains are poor parents and PIs might need to plan to cross foster litters by preparing adoptive mothers. In this case, it must be outlined in an

approved IACUC protocol.

Recommendations for successful cross fostering are below:

Selection of a foster dam:

1. Foster litter should not be more than 48 hours older than the orphaned litter.
2. Foster mothers should either be proven good mothers, or of a strain known to have good maternal care, such as Balb/c, FVB, or Swiss Webster. Most rats are excellent mothers, although a proven mother would be preferred.
3. Ideally, the foster dam and litter should have a different coat color or pattern than the orphan litter to aid in differentiation of the litters.

Steps for successful cross fostering:

1. The chance of success is higher if the pups are 5-7 days old.
2. The number of pups in the litter should not change, and the total number of pups in the litter should not exceed 10.
3. Remove equal numbers of pups as intended to replace with orphaned pups, but avoid removing the foster mother's pups entirely as this may lead to rejection.
 - Humanely euthanize any remaining pups from the orphaned litter and those removed from the foster dam.

Introducing the orphaned litter:

1. Remove the adult male (if present) and house the male separately until weaning. The male may be returned once the litter is weaned.
2. Briefly remove the foster dam to a clean cage.
3. Replace some of the foster dam's pups with orphaned pups
 - Gently rub the orphaned pups with dirty bedding from the foster dams cage to pick up the scent of the foster litter.
 - It is also recommended to rub the new pups on the perineal region of the foster mother to aid in transferring her scent to the new pups.
 - Take care that the orphaned pups do not become hypothermic during the transfer, as this may reduce the chances of the foster mother accepting the orphans.
4. Return the foster dam to the cage with the new blended litter.
5. Return the cage to the rack for the next 2-3 hours, checking on the pups occasionally. Avoid opening the lid as much as possible. Make a note on the cage card.

- If the foster dam appears to have accepted the litter, leave them overnight and check again in about 12 –18 hours.
- If at any point the foster mother removes pups from the nest, is seen dragging pups around, or cannibalizes any pups, the foster attempt has failed, and the remaining orphaned pups should be removed.
- A second cross foster attempt can be made if the orphaned pups are medically stable, otherwise they should be humanely euthanized.

C. Weaning:

Mice and/or rats should be weaned between 19-23 days of age with 21 days of age being the most common age for weaning. Pups may be weaned as early as 17 days of age if death of the dam or the birth of a post-partum estrus litter occurs. Litters younger than 17 days may be weaned only with veterinary medical support in the case of death of the dam, typically on a recharge basis (this may include, for example, soaked chow slurry and/or subcutaneous fluids). Some transgenic, inbred, or specialty strains do not mature as quickly as wild type mice and/or rats and require an extended nursing period. Breeding schemes should be tailored in a protocol to accommodate these strain differences; for example, if a strain requires delayed weaning, remove the male prior to birth so the female does not become pregnant again immediately after parturition. The male may be reunited with the female after weaning. Space requirements for the cage size must be followed at all times.

In cases of delayed weaning, pups are maintained with the dam until they are sufficiently mature to be weaned; in this case a notation needs to be made on the cage card or label. **When strains typically require an older weaning age, this exception should be described in the approved animal care and use protocol and must be discussed with the husbandry staff and facility manager in advance.**

Weanling rodents are separated by sex and housed in a density appropriate for the facility and caging. Best practice when weaning rodents is to provide several pieces of dry chow on the floor to encourage food consumption. When weaning runty rodents, provide a tissue culture (plastic) dish (available in most vivaria) of soaked chow or other hydrating nutritional support and leave a 'special instructions card' on the cage indicating there are runty weanlings present and they should be given soaked chow daily. Failure to do this may result in unnecessarily increased mortality.

If the PI fails to wean a litter or has multiple litters in a cage that is not sized for two litters, not approved in a facility SOP, or in an IACUC approved protocol, husbandry staff will wean and separate the overcrowded cages. A 24 to 48-hour notice may be given to

the PI for overdue weaning. Standard sized cages with multiple litters and/or more than one dam should be separated upon discovery.

When genotyping, the IACUC policy "[Rodent Genotyping and Identification Methods](#)" must be followed. Sampling for genotyping is generally completed prior to 17 days of age per the IACUC policy. Lack of genotyping results is not a valid reason to delay weaning a litter.

D. Record Keeping for all Rodent Colonies:

Breeding records are kept by the PI. Cage specific breeding cards are to be used to track breeding/pairing dates, plug dates (if known), birth dates, and wean dates. These may be required by the facility manager on an individualized or facility wide basis. Individual facilities should develop specific procedures for tracking breeding progress, births, and weaning dates. The Campus Veterinarian's Office can help set up these programs/procedures and tailor them to facility specific needs. Animals born to a breeding protocol are recorded in breeding logs kept by the PI or the colony manager if the facility is maintaining the breeding colony. Animals must be entered into the Animal Tracking System (ATS) when weaned, used experimentally, or when genotyping samples are collected in accordance with an approved animal care and use protocol as described in IACUC policy "[Animal Acquisition, Acclimation and the Tracking System Policy](#)". The ATS should be updated frequently (quarterly at a minimum).

E. Space Coordination:

Prior to setting up any breeders the PI or designated protocol staff must formulate a breeding and space plan with the husbandry staff and facility manager. PIs must maintain their colonies within their allotted space. A facility designated percentage of cages or discreet number allotted for breeding will be left available for weaning and separating litters.

PIs that do not abide by facility procedures or this policy for weaning and separating may lose the privilege of maintaining their own breeding colonies.

IV. Resources:

1. ILAR, Guide for the Care and Use of Laboratory Animals
<http://nap.edu/12910>
2. Wayne State University "Rodent Breeding and Weaning Policy"
<https://research.wayne.edu/iacuc/rodentbreedingandweaning>
3. The Laboratory Rat (Second Edition) American College of Laboratory Animal Medicine (2006)

<https://www.sciencedirect.com/science/book/9780120749034>

4. IACUC Policy-32 "Rodent Genotyping and Identification Methods"
<https://research.ucdavis.edu/wp-content/uploads/IACUC-32.pdf>
5. IACUC Policy-40 "Animal Acquisition, Acclimation and the Tracking System Policy"
<https://research.ucdavis.edu/wp-content/uploads/IACUC-40.pdf>
6. SC-31-100 "Husbandry Care For Non-USDA Rodents"
<https://research.ucdavis.edu/wp-content/uploads/SC-31-100.pdf>