

**Subject: Rodent Overcrowding and Breeding**

Source: Institutional Animal Care and Use Committee (IACUC)  
Effective Date: 05/13/2024  
Replaces: 10/12/2020  
Applies to: Personnel involved in research or teaching studies involving animals  
Reference: Animal Welfare Act; PHS Policy on Humane Care & Use of Laboratory Animals;  
Guide for the Care & Use of Laboratory Animals

**Introduction**

The Institutional Animal Care and Use Committee (IACUC) maintains oversight of federally mandated rules and regulations of animal research for the University of Colorado Denver | Anschutz Medical Campus (CU Denver | Anschutz).

**Policy Statement**

This policy addresses rodent overcrowding, which can negatively affect animal welfare. It is a violation of federal regulations, the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) accreditation guidelines, our Public Health Service (PHS) assurance and CU Denver | Anschutz policy to permit overcrowding. A cage is overcrowded when the number allowed per cage or the weight of the animal in the cage exceeds the CU Denver | Anschutz IACUC maximum cage density for the species which are based on the [Guide for the Care and Use of Laboratory Animals](#) (8<sup>th</sup> Edition). Only the IACUC may grant exemptions to this policy based on scientific justification. For any exemptions, it is the responsibility of the Principal Investigator (PI) or their staff to inform the Office of Laboratory Animal Resources (OLAR). OLAR will verify that the approval has been granted and assist the investigator in the proper labeling of the cage.

**Responsibilities**

- PIs and researchers:
  - MUST ensure that the number of animals per cage and breeding schemes are acceptable per the regulations, AAALAC standards, as well as CU Denver | Anschutz IACUC policy.
  - MUST flag breeding cages appropriately with OLAR provided flags.
  - MUST record breeding colony information, such as accurate birth dates and wean dates, on the “Check for Pups/Wean” cards.
  - MUST monitor their colony frequently enough to prevent overcrowding.
  - MUST perform responsible colony management by weaning litters on time (prior to day 28 OR if a female has a second litter) to prevent overcrowding. Any deviations from this policy can impact animal welfare and potentially be reported to the IACUC and OLAW.
  - MUST ensure that there is sufficient space to wean animals in the room. If adequate vacant space is not available in the housing room, the investigator must discuss this with OLAR.
- OLAR staff:
  - Will monitor the number of animals per cage during daily health checks and record wean dates as applicable.
  - Will identify improperly flagged breeding cages or mixed-sex animals and provide notice at cage level.
  - Will provide research staff until 8 AM of the second business day after being found to correct issues.
  - Will separate cages that require correction at the due date/time and charge \$75 for the service.

**Numbers per Cage**

In order to provide the best welfare for the research animals and to allow investigators the opportunity to perform research without undue restrictions, several breeding schemes are permitted. The investigator should choose the

best breeding scheme for each strain. **Responsible and active colony management is required. Each breeding female is only permitted to have ONE litter that she is raising at any one time in any breeding scheme unless there is an IACUC-approved exemption. Exemptions must have a scientific justification based on data or a request for a case-by-case veterinary exemption for multiple litters per female. Any noncompliance with this policy that is an animal welfare issue, as determined by the veterinary staff, will require immediate corrective action by OLAR staff. Any separation performed by OLAR will result in a \$60 charge per cage. In any breeding cage, TWO or more adult males MUST NEVER be housed together due to the frequent occurrence of inter-male aggression and competition for females.**

For non-breeding cages, there should be no more than:

- 5 adult mice in a standard cage
- 3 adult rats in a multi-species cage
- 3 adult rats weighing <400 grams or 2 animals >400 grams in a static or standard rat ventilated cage

For appropriately labeled breeding cages, the following breeding schemes and numbers are allowed:

- **Monogamous Pair - ONE adult male and ONE adult female**
  - This is the PREFERRED method to prevent overcrowding because it:
    - Offers extended nursing time for inbred strains known to be small and slow growing
    - Maximizes productivity of females by using post-partum estrus
    - Allows for identification of the dam for the litter for more accurate breeding records
  - Only ONE litter per female per cage is allowed at any given time
    - If a second litter is found, the cage will be flagged for weaning by 8 AM the second business day after the second litter has been found.
  - Male is permitted to remain with the female at all times provided that the lab actively manages their colony and weans litters on time.
  - If the older litter does not appear to be ready to be weaned, the investigator can request that the animals be examined by a veterinarian or veterinary technician. If it is determined that the older litter should stay with the mother, the cage will continue to be monitored for animal welfare concerns until the older litter is ready to be weaned. For strains when the older litter routinely requires additional time with the mother, using the post-partum estrus for managing the breeding colony should not be used and the male should be removed prior to the female littering.
- **Breeding Trio – ONE adult male and TWO adult females**
  - Trio breeding must be approved on the IACUC protocol.
  - This method of breeding may be beneficial for certain strains where the female produces small litter sizes or benefits from female co-parenting.
  - There cannot be more than 14 pups total in the cage.
    - When two females have litters resulting in more than 14 pups, the cage will be monitored by the veterinary staff for animal welfare concerns. The cage will be kept intact and litters will wean on-time, unless there is a welfare concern. If the lines that are being bred as trios consistently produce more than 14 pups between two females, then the PI will be required to breed via monogamous pairs.
  - Three adults may be housed in the cage until the first litter is born, which requires one of the adults be removed by 8 AM on the second business day. This will allow the female to be bred via post-partum estrus.
    - If a litter is found, the cage will be flagged by OLAR staff with the date that one of the adults must be removed by. If the lab does not remove one of the adults by this deadline, then OLAR staff will perform the separation. The default will be the removal of the male to ensure that the female who is the mother of the litter remains with the pups and to allow for the benefit of aunting behavior.
    - If a second litter is found in the cage prior to the removal of the adult male, the initial deadline written on the flag must still be met for the separation of the male.

- Triad/Harem Breeding must be indicated on the “Wean/Check for Pup” flag that is placed on the cage.
  - Improperly flagged Triad/Harem breeding schemes will be flagged for correction. If not corrected by 8 AM of the second business day, the male will be removed from the cage and any pregnant females will be separated into their own cages.
  
- **Harem Breeding – ONE adult male and up to FOUR adult females**
  - This scheme is **HIGHLY** discouraged due to the potential for severe overcrowding.
  - This scheme will only be approved for protocols that are producing time-pregnant embryos and not for producing live pups.
  - All cages using this breeding scheme must be indicated on the “Wean/Check for Pup” flag and posted at cage level. If Harem is not indicated on the flag, the cage will be flagged for correction by 8 AM the second business day after being found. If correction to the flag is not made by the deadline, the females will be separated from the male so that there are no more than two females per cage. Any pregnant females will be placed into their own cages prior to littering.
  - Females **MUST** be checked daily for plugs and separated immediately from the cage if a plug is found.
  - If the male remains with the females with no evidence of plugs, all females must be checked daily for pregnancy 14 days after the animals are placed together in case plugs were missed. Pregnant females must be removed from the cage and placed in their own cage prior to littering.
  - Any litters found in this breeding scheme will be reported to the IACUC and may lead to the removal of this breeding scheme from the protocol.

### Procedures

- Separations performed by OLAR for overcrowded/mixed-sex cages as defined will incur a \$60 charge to the PI.
- Improperly flagged cages or potentially mixed-sex animals will be flagged with the “Overcrowded Notice”.
- Cage flags will be the only notification to the PIs for overcrowded or mixed-sex cages. OLAR will not send email notifications regarding overcrowded cages.
- Animal care providers will place a completed “Overcrowded Notice” flag on the cage with the date that the overcrowding must be corrected by. The PI will have until 8 AM on the second business day to correct the situation or OLAR staff will separate the animals and charge a fee.
- Severely overcrowded cages
  - Cages that have more than twice the maximum number of animals permitted are reported to a veterinarian as an animal welfare concern. They will be separated immediately if the PI cannot be contacted. Ultimately, concern for animal welfare overrides investigator notification.
- Breeding Cage Set-up
  - All breeding cages **MUST** have a “Check For Pups / Wean” flag placed on them. If utilizing the Trio or Harem breeding scheme, indication on the flag is required.
  - Breeding cages will be checked during the normal health check for new litters. Wean dates will be calculated 21 to 28 days from date of birth and written on the flag. Cages with “DO NOT DISTURB” flags will be handled per the Do Not Disturb Policy.
  - It is the responsibility of the first person that discovers a new litter (animal care tech, veterinary tech, PI staff) to write the date of birth and weaning range on the “Check For Pups / Wean” flag.
- New litter age determination
  - For a litter not present the day before (most likely born at night), the day it is found will be recognized as the date of birth and day 0.
  - If physical developmental characteristics clearly support a litter is older than day 0 when first found, rodent aging charts are used to provide the most accurate estimated date of birth.
  - The number of animals born must be written on the back of the “Check For Pups / Wean” flag for appropriate accounting of all animals when submitting the cage card request form.

- Weaning litters
  - The typical weaning age of mice is 21 days. However, due to the large number of genetically modified mice that need to remain with their mothers for longer than 21 days, separation of weanlings is permitted to occur between 21-28 days after the day of birth.
    - If a new litter is found on a Monday, the 21-28 day weaning range dates would both also occur on Mondays.
  - Mice must be weaned by day 28 of the wean date range unless there is an IACUC exemption on the protocol or a veterinary exemption is requested for animal health.
    - An animal care staff member, veterinary technician, or researcher can request a veterinary exemption. Veterinary staff will evaluate the animals and determine if weaning can be delayed and if so, for how long.
- Litters that have not been weaned by day 29 will be separated on that date by OLAR staff and will be charged the \$60 fee.
  - The “Check For Pups/Wean” flag will be considered the notification for the investigator. OLAR will not send e-mail notifications as reminders to wean.
  - In advance, investigators can contract with OLAR to wean or separate animals for the normal technical hourly rate or transfer their colonies to the OLAR run Breeding Core.
- Placing cage cards
  - Per IACUC policy, the research staff must immediately label cages that have been weaned or separated with appropriate identifying information per the IACUC Policy and submit the cage card request to the OLAR office.
  - Cage card request form must be completed filled out and include the total number of pups born in the litter including those weaned and those euthanized prior to weaning.
  - The PI has up to 3 business days to place the cage cards. Cages found without cage cards will be marked for the date and time. If cage cards are not placed on the cage within 3 business days, per the IACUC Policy, there will be a \$5 fee per cage to place the cage cards.
  - Reusing cage cards is not permitted, new cards must be ordered for all separations and weaning events. Ordering new cards ensures that animal numbers are properly reduced from the allowable number of animals on the protocol.

Exceptions to this policy will be considered by the IACUC on a case by case basis on presentation of adequate scientific justification.

Per regulatory requirements, failure to comply with this policy may result in notification of your funding agency (e.g. NIH) and regulatory agencies (e.g. USDA) that your research has violated federal and/or local policies regarding the humane use of animals. This notification may affect continuous funding of your animal-related research. Further, depending on the violation, you may be required to take additional training and/or your privilege to conduct animal research at UC Denver might be temporarily suspended or even completely revoked.