



## IACUC Rodent Breeding Policy

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### 1. Overview

#### Purpose

Drexel University Institutional Animal Care and Use Committee (IACUC) has established this policy to provide information for the proper maintenance of rodent breeding colonies, specifically mice (*Mus musculus*) and rats (*Rattus rattus*).

### 2. Definitions

**Pair breeding:** Breeding of one (1) adult male and one (1) adult female.

**Trio breeding:** Breeding of one (1) adult male and two (2) adult females. This must be justified and approved in the protocol.

**Harem breeding:** Breeding one (1) adult male and three (3) or more adult females. This exception must be justified and approved in the protocol.

**Post-partum estrus (continuous breeding):** Female rodents may undergo a fertile estrus 14-24 hours following parturition. Females that are housed with males when they give birth can therefore become pregnant with a second litter that would be delivered around the time of the weaning date of the first litter. This can lead to overcrowding if the weanlings are not separated at 21 days of age. This exception must be justified and approved in the protocol.

**Female/Female co-parenting:** Two (2) females in a cage rearing a litter. This exception must be justified and approved in the protocol.

**Pup:** Neonatal rodents up to 21 days of age; a group of pups from the same mother is called a **litter**.

**Weanling:** Young rodent recently separated from its mother; usually 21 - 23 days old.

**Weaning:** The moment pups are transferred out of the mothers' cage.

**Overcrowding:** More than five (5) mice per cage that are 30 grams in weight or less or a litter over 23 days of age.

### 3. Breeding Procedures

1. All breeding procedures must be described in an approved IACUC protocol. Any deviations or changes from the breeding scheme must be approved in an amendment submitted to the IACUC prior to implementation of any of these changes. Amendment forms should be submitted through DragonSpot.
2. Protocol should include:
  - a. Section E, especially part 2: Predicted numbers of animals generated based on breeding strategy, predicted numbers of animals that can be used for experiments based on genotypes needed (keeping in mind whether predicted genotypes will be generated according to Mendelian genetics).



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- b. Section H: Breeding scheme, weaning age, duration that breeders are used, method for obtaining tissue samples for genotyping, method for identifying pups (ear punch, toe clips, animal ID tags, or tattoo)
  - c. Any other pertinent information related to breeding procedures.
3. The standard breeding set up is pair breeding. The male rodent must be removed prior to the female giving birth. The female should remain in the original breeding cage and the male should be single housed in a clean cage. If breeding during post-partum estrus is approved in the breeding protocol, provisions to ensure that cages do not become overcrowded must be in place. This must be described and approved in the IACUC protocol. Simultaneous presence of two or more litters in a single cage is not permitted, unless scientifically justified and approved in the IACUC protocol.

Trio breeding schemes must be approved in an IACUC protocol. Justification must be provided for this increased density, including a description of management techniques that allow pups to develop to weaning age without detrimental effects for the mother or litters.

NOTE: To avoid overcrowding, pregnant females must be separated into individual maternity cages for delivery of litters. The IACUC recommends separation of pregnant females up to one week before expected delivery. Maternity cages must be closely observed for delivery of pups and the date of birth recorded on cage cards. Simultaneous presence of two or more litters in a single cage is not permitted, unless justified and approved in the IACUC protocol.

4. Enrichment must be provided for every animal cage unless scientifically justified and approved in the IACUC protocol. Two forms of enrichment are required for singly housed animals. (See IACUC Policy for Animal Enrichment on the [IACUC website](#))
5. All litters must be weaned between 21 – 23 days of age. Pups must be separated by sex into individual male or female cages, keeping in mind that **housing density should not exceed 5 animals/cage**. Exceptions to weaning age (beyond 23 days) must be reviewed and approved by the IACUC. A Weaning Calendar is provided at the end of this policy document and on the IACUC website under Policies and Procedures.
6. Breeding males should be singly housed after being separated due to their aggressive nature after breeding. Breeding males may cause grave physical harm or death to a male cage mate if cohoused.
7. Cage Identification
  - All breeding cages **MUST** be marked using the purple plastic breeding alert cage flag provided by ULAR. (See Appendix A of this Policy for example)
  - Investigators may create their own cage cards. Suggested templates are available for convenience on the IACUC website under “[Policies and Procedures](#)” and can be modified, as needed.



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- Horizontal and Vertical templates are available.
- Example: These templates can be printed on standard 2"x4" mailing labels and placed on standard 3"x5" index cards.
- If the PI/staff chooses to make breeding cards the following information must be included:
  - a. Protocol Number
  - b. Name of PI
  - c. The number of females in the cage
  - d. The date the breeding cage is set up
  - e. Date of birth of litters (DOB)
  - f. Weaning date

### 4. IACUC Recommendations

The recommendations below are guidelines to help ensure healthy colonies. Researchers should contact ULAR staff or the IACUC if they have any questions or concerns regarding their breeding colony.

1. **Monitor breeding cages and weaning pups.**
2. **During Pregnancy.** Allow animals to be co-housed until signs of pregnancy are noted, i.e., swollen abdomen of females (usually seen 14 – 18 days after initial breeding setup).
3. **Housing newly weaned pups.** Cages should contain enrichment, and accessible food and water. Providing longer sipper tubes if using water bottles (provided by ULAR) is recommended.
4. **Post Weaning.** Adult females may be placed into a breeding cage with a male for production of another litter.
5. **Additional Monitoring.** A specific genetic modification that routinely results in a longer period before weaning should be noted in the IACUC protocol. Any changes or updates to approved breeding procedures must first be submitted as an amendment for review and approval by the IACUC.
6. **Contact Veterinary Staff.** Any mouse pups weighing less than 10 grams at 23 days must be reported to veterinary staff for evaluation. If any other animal care concerns are noted, please contact ULAR for additional guidance and assistance.
7. **Retirement of Breeding Rodents.** Fertility of rodents is highest during the first 6 months of their lives. After a year of age, fertility is very low. Therefore, the IACUC recommends retiring breeders between 8 to 12 months of age. Individual genetic lines will vary, and investigators must use their best judgment and experience to decide when to replace breeders.



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## 5. Quality Control and Compliance

### 5.1 Adherence to IACUC protocol

All breeding procedures must be approved in an IACUC protocol. Any deviation from the approved breeding scheme must be approved in an amendment submitted to the IACUC prior to any change being made. Amendment forms should be submitted through DragonSpot.

### 5.2 Breeding Reports

All in house births, including all embryos greater than E16 and all live births, even if the animals are euthanized prior to weaning, must be reported monthly to ULAR for maintaining approved animal numbers on each IACUC protocol. An In-House Birth Report must be submitted monthly, even if no animals were produced during the month. The report should be submitted by the 5<sup>th</sup> of the following month. The In-House Birth Report is an online form and can be accessed using the link provided: [In-House Birth Report Form](#) and is available on the IACUC website under “Policies and Procedures”.

Any questions can be sent to [ULAR@drexelmed.edu](mailto:ULAR@drexelmed.edu).

### 5.3 Overcrowding

If significant overcrowding is noted by ULAR veterinarian or ULAR staff, or if a litter is more than weaning age (23 days old), a “**Please wean/separate**” card will be posted on cage by ULAR animal care staff with the current date. Research staff will be notified and are responsible for weaning and/or separating the animals within two (2) calendar days of this posting. ULAR husbandry staff will wean and/or separate the animals on the third day after notification and the PI will incur charges at the current hourly rate for the technical staff providing this service.

A repeated instance of overcrowded status is considered a non-compliance with this policy. Noncompliance can be prevented with regular consultation with ULAR animal care staff regarding any breeding issues noted in your colony, and timely weaning of animals as described in the approved IACUC protocol and this policy. Every effort should be made to resolve breeding related issues prior to reporting these issues to the IACUC.

### 5.4 Reporting Noncompliance and Animal Welfare Concerns

Continued findings of non-compliance with this policy, or incidences of overcrowding cages to the extent that animal welfare is comprised will be reported to the IACUC for further review and resolution.

## 6. Responsibilities

### 6.1 Drexel University IACUC Responsibilities

The Drexel University IACUC and the IACUC Office are responsible for maintaining this guidance document, training, and monitoring. All exceptions to this policy must be approved by the IACUC. For inquiries regarding these procedures, please contact the Director of Animal Welfare, as part of the Office for Research & Innovation (ORI) or the Attending Veterinarian, as part of University Laboratory Animal Resources.



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### 6.2 Principal Investigator Responsibilities

Principal investigators (PI), their designated staff and University Laboratory Animal Resources (ULAR) staff who are managing rodent breeding colonies under an approved IACUC protocol are required to adhere to this policy.

**It is the PI's responsibility to plan colony management, including, but not limited to timely weaning of litters and prevention of overcrowded cages.** PI's must ensure that an efficient communication method is in place for notification from husbandry and veterinary staff regarding animal issues.

## 7. Resources

**Strategies for Maintaining Colonies of Laboratory Mice: A Jackson Laboratory Resource**  
Manual Ray Lambert (Sr. Editor) 2009.

### Drexel University IACUC Weaning Calendar

DOB	Wean on (21d)	Wean on (28d)	DOB	Wean on (21d)	Wean on (28d)	DOB	Wean on (21d)	Wean on (28d)	DOB	Wean on (21d)	Wean on (28d)	DOB	Wean on (21d)	Wean on (28d)	DOB	Wean on (21d)	Wean on (28d)
1-Jan	22-Jan	29-Jan	1-Feb	22-Feb	29-Feb	1-Mar	22-Mar	29-Mar	1-Apr	22-Apr	29-Apr	1-May	22-May	29-May	1-Jun	22-Jun	29-Jun
2-Jan	23-Jan	30-Jan	2-Feb	23-Feb	1-Mar	2-Mar	23-Mar	30-Mar	2-Apr	23-Apr	30-Apr	2-May	23-May	30-May	2-Jun	23-Jun	30-Jun
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31-Jan	21-Feb	28-Feb				31-Mar	21-Apr	28-Apr				31-May	21-Jun	28-Jun			



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1-Jul	22-Jul	29-Jul	1-Aug	22-Aug	29-Aug	1-Sep	22-Sep	29-Sep	1-Oct	22-Oct	29-Oct	1-Nov	22-Nov	29-Nov	1-Dec	22-Dec	29-Dec
2-Jul	23-Jul	30-Jul	2-Aug	23-Aug	30-Aug	2-Sep	23-Sep	30-Sep	2-Oct	23-Oct	30-Oct	2-Nov	23-Nov	30-Nov	2-Dec	23-Dec	30-Dec
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31-Jul	21-Aug	28-Aug	31-Aug	21-Sep	28-Sep				31-Oct	21-Nov	28-Nov				31-Dec	21-Jan	28-Jan

## 8. Revisions

Edition 001/Effective Date: 3/2/2016 - Original Document

Edition 001/Review Date: 09/2021

Edition 002/Revision and Effective Date: 05/22/2023 - Revised Document.

Updated Mouse Breeding Policy to become a Rodent Breeding Policy to include rats, *Rattus rattus*. Revision to breeding report submission procedures. Added Weaning Calendar.