

MOUSE BREEDING CONFIGURATIONS

1. Policy statements

This policy outlines the different mouse breeding configurations allowed in NUS. In accordance with the Guide, NUS standard mouse cages are only large enough to house **3 adults plus 1 litter**. NUS Animal Care and Use Committee (IACUC) will allow a maximum of 3 adults and 1 litter per breeding cage regardless of litter size.

2 Definitions

Overcrowding	Housing density that exceeds what is stated in this policy, unless an approved exemption is obtained from NUS IACUC
Pair Breeding	Pair breeding consists of 1 female mouse and 1 male mouse housed in the same cage. <u>Only one litter can be housed in each cage.</u>
Trio Breeding	Trio breeding consists of 2 female mice and 1 male mouse housed in the same cage. <u>Only one litter can be housed in each cage.</u>
Co-rearing	Co-rearing consists of 2 dams with up to 2 litters housed in the same cage that are 3 or less days apart.

3 Scope

This policy applies to the breeding of all mouse strains in NUS.

4 Guidelines

4.1 Principles

A maximum of 3 adults and 1 litter per breeding cage will be allowed in NUS regardless of litter size. This is an exemption to the National Advisory Committee for Laboratory Animal Research (NACLAR) Guidelines and is based on the following considerations:

1. Standard CM cages have a floor space of 554cm².
2. The Guide for the Care and Use of Laboratory Animals, 8th Edition (2011) indicates that 330cm² floor space is required for a dam with a litter plus 96.7cm² for each group-housed mouse > 25g. Thus 3 adults plus 1 litter requires 523.4cm².
3. NACLAR Guidelines (2004) does not mention a dam with a litter. However, it indicates that a floor space >96 cm² is required for each mouse >25 g, and each mouse <10 g should have an additional 38cm². This equates to 3 adults and 7 pups.

4.2 Weaning

The standard practice of weaning is to do so at 21 days of age, at which time mice are separated by sex into not more than 5 animals per cage.

NUS standard mouse cages can house a maximum of 5 adults per stock cage.

4.3 Standard Breeding Configurations

Principal Investigators are to maintain proper breeding records, in accordance with the NUS Policy for Rodents Breeding Colony.

1. Pair Breeding

Pair breeding consists of 1 female mouse and 1 male mouse housed in the same cage. Only one litter can be housed in each cage.

2. Trio Breeding (formerly termed “Approved trio breeding”)

Trio breeding consists of 2 female mice and 1 male mouse housed in the same cage. Only one litter can be housed in each cage. In order to avoid having two litters in the cage, the second female must be removed when she is visibly pregnant, if one litter is present in the cage. A second female may remain as an “aunt” for as long as she is not visibly pregnant.

4.4 Exceptions to NUS Standard Breeding Configurations

The NUS IACUC may grant exceptions to this policy, but only after specific review and approval of the scientific justification (i.e. breeding records must be provided to the IACUC). Alternatively, when not approved in the protocol, an exception may be approved on a case-by-case basis (as a medical case) following CM veterinary advice and must be covered under clinical care.

Common exceptions that require protocol specific approval are included in the points below.

4.5 Co-rearing

Co-rearing consists of 2 dams with 2 litters housed in the same cage that are 3 or less days apart.

Acceptable justifications may include:

1. High anxiety strains or stocks that have documented evidence of poor mothering (increased cannibalism, failure to nurse as evidenced by small or absent milk spot(s), not attending to pup(s), pup(s) scattered around cage, etc.).

First time mothers are often able to rear subsequent litters successfully and the IACUC may only approve co-rearing if records for subsequent litters show poor mothering.

The male must be removed once both females are noted to be pregnant, or when one female gives birth (regardless of whether the other female is observed pregnant).

If litters are born more than 3 days apart, the females and their litters must be separated in order to prevent trampling of the younger litter when they become ambulatory.

4.6 Extended Weaning

1. Weaning can be performed at 28 days of age for strains with pups that do not consistently weigh >8g at 21 days of age.
2. There can only be 1 litter in the cage.
3. If more than 1 litter is present and the cage contains:
 - a. One dam: any pup > 8 g must be weaned when the new pups are born.
 - b. Two dams: the older litter must be weaned or separated with the respective dam

4.7 Documentation and Labelling Requirements

For any strain or stock for which exceptions to this policy have been approved, the following are required to ensure that performance standards are monitored and met:

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1. The cage must be labelled with the specific exception e.g. Co-rearing and Extended Weaning, as directed by CM.
2. The PI must submit, upon request, breeding records to the IACUC and/or CM Veterinarians. At a minimum, the information must include:
 - a. Number of pups born, date of birth, and number weaned per litter;
 - b. Weight of pups at weaning (for extended weaning exceptions)
 - c. Morbidity of pups and breeder(s) i.e. any clinical issues
 - d. Mortality of pups and breeder(s)

4.8 Overcrowded cages

Any cage found with breeding configurations not mentioned above, not approved in the protocol, and/or improperly labelled, will be considered overcrowded. CM will adhere to the NUS IACUC Policy Animal Housing Density and separate animals as appropriate.

5. References

1. [Guide for the Care and Use of Research Animals, Eighth Edition. National Research Council; National Academy Press, Washington, DC. 2011](#)
2. [Guidelines on the Care and Use of Animals for Scientific Purposes. National Advisory Committee for Laboratory Animal Research. 2022](#)
3. [The Jackson Laboratory.](#)
4. Cornell University. Rodent Husbandry and Breeding.
5. McGill University. Mouse Breeding Colony Management.
6. Duke University. Cage Space Requirements for Mice.

6 Related documents/SOPs

1. [IACUC Policy P205 ANIMAL HOUSING DENSITY](#)
2. [IACUC Policy P207 POLICY FOR RODENT BREEDING COLONY](#)
3. [CM training slides for IACUC Policy on Mouse Breeding Configurations](#)

Revision	Author	IACUC approval date
P206.. Created to define the different breeding configurations allowed in NUS	Dr Lois Zitzow	16 August 2019
P206.01. Revisions to: <ol style="list-style-type: none"> i. clarify section 4.5 "Co-rearing" that litters 3 or less days apart can be housed in the same cage ii. attached CM training slides for the policy in section 6 iii. Remove Annex A 	Dr Chew Li Li Dr Lois Zitzow Dr Yasmina Paramastri Dr Jassia Pang	16 September 2019
P206.02. Reviewed to: <ol style="list-style-type: none"> 1. ensure policies are in compliance with the NAELAR Guidelines 2022 and the updated Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004; and 2. up-to-date with current practices Changes made under section 5, updated to "Guidelines on the Care and Use of Animals for Scientific Purposes. National Advisory Committee for Laboratory Animal Research. 2022"	Dr Chew Li Li IACUC	3 January 2023
P206.03. Revised to remove outdated para 4.7 Alternative Trio Breeding (for protocols approved prior to August 2019)	Dr Chew Li Li	21 July 2025