

**Arizona State University
Institutional Animal Care and Use Committee
STANDARD INSTITUTIONAL GUIDELINE**

MOUSE BREEDING

ASU has established mouse breeding guidelines in order to serve 3 critical aims: (1) to be in compliance with the *Guide for the Care and Use of Laboratory Animals*¹ as well as the position statements on mouse breeding of both the Office of Laboratory Animal Welfare (OLAW)² and AAALAC International³, (2) to support the well-being of the mice, and (3) to enable investigators to have efficient and effective production of animals to accommodate their research needs. These guidelines take into account the required provisioning of both minimum housing space and a social environment for social species. The below guidelines were created to ensure that cages (1) enable animals to make normal postural adjustments, (2) enable animals to rest away from soiled areas, (3) provide free access to food and water, (4) provide sufficient space for mothers with litters to enable the pups to develop to weaning without detrimental effects to the mother or the litter, and (5) provide a favorable social environment for mice with the understanding that, for scientific reasons or for the well-being of the mice, certain breeding situations require an adult mouse to be housed alone for a period of time.

Standard Housing Options for Breeding Mice:

Permanent pairing:

1. One male may be permanently housed with one female in a standard “shoebox” cage (67-70 in²).
 - a. Permanent pairing increases reproductive frequency by taking advantage of the female’s postpartum estrus that occurs approximately 24 hours after giving birth.
 - b. Females that become pregnant during their postpartum estrus will give birth to their next litter approximately 20-24 days after the birth of the first litter. Therefore, it is highly recommended that offspring produced in cages containing a permanent pairing be weaned by 21 days of age so as not to be present in the breeding cage when the next litter is born.
 - i. Birth of a second litter while the first litter is still in the cage will often lead to the death of the new litter due to its inability to compete for the female’s milk and being trampled by cagemates.
 - ii. Therefore, the presence of sequential litters in a cage is an issue that should be avoided or, if it does happen, addressed immediately.
 - c. Postpartum breeding can be physiologically demanding on the female as she is continually lactating and gestating. As a result, the female’s reproductive life may be shortened, and pups may acquire less nutrients during development.

Temporary pairing:

1. It may be preferable to remove the male from the female’s cage once the female is determined to be pregnant (e.g., presence of a mating plug or abdominal distention). Doing so:
 - a. Puts less physiological demand and reduces potential stress on the female. Either of these can lead to the female cannibalizing her offspring.
 - b. Eliminates the chance of sequential litters existing in a cage as a result of postpartum mating and therefore enables pups to be kept with their mother longer (up to 28 days), which can be advantageous for many slow-developing strains and transgenic lines.

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- c. Eliminates the possibility that the male may cannibalize the pups or interfere with maternal behaviors, which can impact the well-being of the offspring.
 - d. Enables one to carefully time mating to produce offspring near a specific date.
2. As a result of temporary pairing, the adult mice will temporarily need to be housed singly.
 - a. Females that are confirmed pregnant can be housed singly until the birth of their litters.
 - b. Breeder males may be singly housed for periods between breeding. Housing of breeder males with other males typically leads to fighting, and therefore co-housing adult breeder males is not an option.

Purchase of time-pregnant females:

1. When a female rodent is ordered time-pregnant, she can be housed singly upon arrival until the birth of her litter.

Alternative Housing for Mouse Strains that are Less Fecund:

1. In some instances, it may be beneficial to both reproductive output and animal well-being to co-house two breeding females in a standard shoebox cage (67-70 in²).
2. Reasons for housing two breeding females together include:
 - a. Small average litter size
 - b. Poor maternal care/nurturing
3. When two breeding females are co-housed, the male may NOT be left in the cage during the rearing of the offspring. Instead, the male should be removed once the female(s) are confirmed to be pregnant. Removing the male prior to birth of the litter:
 - a. Reduces the density of animals in the cage and thus reduces waste accumulation during the lactation period.
 - b. Prevents the male from cannibalizing the litter.
 - c. Reduces activity in the cage, and thus the chances of pup trauma resulting from the male trying to breed the dam during her post-partum estrus.
4. Removing the male after pups are born is not desirable.
 - a. It will require disturbing the cage during the neonatal period when the mother is establishing her bond with her offspring and could lead to nest desertion or cannibalism.
 - b. The male may impregnate the female during her post-partum estrus. If this occurs, it is vital that the initial litter be weaned before the birth of the second litter, which may not be possible for slowly developing strains.
5. Separating the male during the lactation period and, thus, bypassing the post-partum estrus tends to extend the breeding life of the females and better enables one to synchronize the breeding of both females in the cage to minimize the age gap between two litters in a cage.

Weaning:

1. Mice should be weaned at 3-4 weeks (21-28 days) of age, with the appropriate weaning age being dependent on the development of the offspring.
 - a. At the time of weaning, the pups should be robust, have open eyes, have ears up, be able to ambulate supported well by their limbs, and have adult fur rather than the sparser, shorter fur of young developing mice.

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- b. Weanling mice must be able to access both food and water.
 - c. Mice of standard strains typically attain these traits by day 21, but pups of some strains and many transgenic lines may not reach this level of development until later.
 - d. All mice should be weaned no sooner than 19 days and no later than 28 days after birth.
2. Allowing a young mouse to develop further prior to weaning is advantageous to its immediate well-being as well as its adult behavior^{4,5}.
 - a. However, waiting to wean the litter poses added risk of cage sanitation issues, as waste accumulation builds up more rapidly as offspring grow and begin feeding and drinking on their own.
 - b. The risk of excessive accumulation of waste prior to weaning is greater when litters are weaned at an older age, litter size is large, or multiple litters are present in the cage.
3. Weaning by 21 days of age ensures that a given female will not have a second litter before the current litter is weaned.
4. If more than 10 days separates the ages of two litters in a cage, the younger litter is at a high risk of runting and even mortality as a result of inadequate acquisition of milk or trampling by the other pups.
 - a. To avoid these negative consequences:
 - i. The older litter needs to be weaned if they are sufficiently developed.
 - ii. If the litters are produced by the same female and the older litter is not sufficiently developed to be weaned, one of the litters needs to be culled or moved to a surrogate female.
 - iii. If two females are housed in a cage, an expectant female should be separated into a different cage if the other female is already nursing a litter that is a week old or more. Preventing a problematic situation is always better than correcting one.
 - iv. If two females are housed in a cage and there are two litters present that are more than 10 days apart in age, the two females should be promptly separated, and the offspring split between the females so that the older litter is with one female and the younger litter is with the other female.
 - v. When breeding one male with two females in a cage, one can avoid having litters being born 10 or more days apart by introducing the male to non-lactating, fully mature (or similarly aged) females so that they become pregnant at similar times. Again, it is best to aim to prevent problems.
5. If the total number of offspring, regardless of relative age between litters, becomes too high to effectively maintain a clean cage environment (based on the assessment of DACT staff, but typically more than a total of 12 offspring over 7 days of age), the number of mice in the cage should be reduced through weaning, culling, fostering, or separating females and their litters as appropriate for the specific situation.
6. DACT vivarium staff will notify labs of the need to wean or separate mice for the following reasons:
 - a. Excessive waste accumulation in a cage
 - b. Overcrowding (typically a cage containing two females and more than a total of 12 offspring over 7 days of age)

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c. Litters over 28 days of age
d. Two litters in a cage that are more than 10 days apart in their ages

Investigators will typically have 48 hours to address the situation, after which the DACT staff will correct the issue by separating the mice as necessary (see the DACT SOP on Overcrowded Rodent Cages for details).

7. Notifications to wean mice should not be part of the standard colony management plan. It exists as a back-up in case the lab misses a needed weaning. If DACT staff needs to provide weaning notifications, then the PI may be charged for the time required to observe and report the needs for weaning. Charges will be at the standard DACT staff labor rate.
8. In some circumstances, if the DACT staff feels that mice are at immediate risk of serious injury or death (e.g., new litter born when a near-weaning age litter is already present), the investigators are expected to address the issue as soon as possible. In these cases, regardless of the timeframe, the DACT staff can decide that waiting any longer for laboratory personnel to address the issue presents excessive risk and will thus perform any needed action (e.g., separation of weanable mice or separating two moms with litters).
9. Principal Investigators will be charged at the standard DACT labor recharge rate for any actions DACT takes to address problematic cages.
10. Mice should be weaned into same-sex groups of no more than 5 mice per cage. Laboratories may be allowed to house mix-sexed weanlings that are less than 5 weeks of age in groups of up to 8 individuals per cage while waiting for genotyping results. This is only permissible if the laboratory reliably separates the weanlings into single sex groups of no more than 5 mice per cage within 5 days of weaning or by the time the oldest mouse in the cage is 30 days of age, whichever comes first. Delays in separation can lead to excessive waste production, fighting among males, and unintended early pregnancy in females, so are unacceptable, and colonies where this occurs will be required to have mice weaned into same-sex groups of no more than 5 individuals.

Resources:

1. *Guide for the Care and Use of Laboratory Animals, 8th Ed.* (2011) National Research Council, Washington D.C.
2. Office of Laboratory Animal Welfare website, Frequently Asked Questions (FAQ) – F10 and F16. (<https://olaw.nih.gov/faqs#/guidance/faqs?anchor=10599>)
3. AAALAC International website, Frequently Asked Questions (FAQ) – Trio Breeding (<https://www.aaalac.org/accreditation-program/faqs/#Ctrio>)
4. Richter, S. H., Kästner, N., Loddenkemper, D. H., Kaiser, S., and Sachser, N. 2016. A time to wean? impact of weaning age on anxiety-like behaviour and stability of behavioural traits in full adulthood. *PLoS ONE* 11(12): e0167652. doi:10.1371/journal.pone.016765.
5. Kikusui, T., Isaka, Y., Mori, Y. 2006. Early weaning deprives mouse pups of maternal care and decreases their maternal behavior in adulthood. *Behavioural Brain Research* 162: 200-206.

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