UBC ANIMAL CARE COMMITTEE

Guidelines for Management and Maintenance of Rodent Breeding Colonies

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1. PURPOSE:

The purpose of this guideline is to accompany The University of British Columbia Animal Care Committee (UBC ACC) Policy 013 on Rodent Breeding Colony Management in order to help researchers to manage and maintain colonies of breeding rodents to ensure the best possible care and welfare of breeding rodents and their offspring.

2. SCOPE:

This guideline applies to all breeding animals (adults and offspring) housed in UBC animal facilities, as well as those facilities that reside at affiliated research institutes, centres and hospitals and that fall under the review of the UBC ACC ("UBC animal facilities").

3. DEFINITIONS:

Archiving Methods: Methods used to archive genetic strains. These include cryopreservation of embryos, spermatozoa, oocytes, or ovarian tissue.

Breeding colony: Group of breeding animals used for the production of research animals that are not available commercially or require local production. The colony includes parents, offspring and replacement breeders.

Genetically engineered (GE) animal: Refers to any animal that has had a random or targeted change in its nuclear or mitochondrial DNA (addition, deletion or substitution of some part of the animal's genome) achieved through a deliberate human intervention (CCAC 3Rs Microsite). Genetically engineered animals also include offspring that result from intercrossing unique genetic strains (e.g. Cre-lox, CRISPR-Cas).

4. BREEDING SCHEMES

Breeding schemes and mating systems should take into consideration the performance based criteria of the species/strain. These schemes may vary according to the needs of the investigator and species. The scheme must be described in the ACC Breeding protocol.

Acceptable breeding schemes for mice and rats include:

A. Paired/monogamous

B. Trio
C. Harem

A. Paired/Monogamous:

1Female (F):1Male (M)
One of the most commonly used methods.
The male and female can be:

1. Left together permanently.
   - Previous older litter must be weaned before the next litter is born.
   - Euthanasia of the new litter prior to weaning of the older litter is not permitted
2. Separated: Before the female gives birth ("on/off" breeding).
3. Separated: After the female gives birth – this takes advantage of post-partum estrus.

Because a male may kill the pups: the male and female must not be recombined until after the litter has been weaned.

Pairs are often used to maintain pedigree (backcrossing or intercrossing) and are useful with regards to limiting surplus animals (see Policy 013 statement #7) by utilizing "on/off" breeding. Males from pair cages can be utilized in rotation mating when large numbers of similarly aged animals are required.

B. Trio:

2F:1M

There are three options for grouping once pregnancy is detected:

1. One of the females remains in the cage with the male continuously. The second female is removed prior to either female giving birth.
2. The two females are left to give birth in the same cage. The male is removed prior to either female giving birth.
3. Both females remain with the male continuously as long as this does not result in overcrowding (see below).

Because a male may kill the pups:

1. The male and females must not be recombined until after all litters have been weaned.
2. If the females are pregnant, the females/male can only be recombined if the females are at similar stages of pregnancy.

Trios do not maintain pedigree unless one female is removed, allowing identification of the dam for each pup. Rotation mating is hard to utilize when the females are left together as you have to wait until the 2nd litter is weaned.

C. Harem:

3-5F:1M

Harem breeding is typically used only for mice. There are three options for grouping once pregnancy is detected:

1. One of the females remains in the cage with the male continuously. Additional females are removed prior to any female giving birth.
2. Two females are left to give birth in the same cage, the male (and any additional females) are removed.
3. Two females remain with the male continuously as long as this does not result in overcrowding (see below). Additional females are removed prior to any female giving birth.

Because a male may kill the pups:

1. The male and females must not be recombined until after all litters have been weaned.
2. If the females are pregnant, the females/male can only be recombined if the females are at similar stages of pregnancy.

Similarly to trios, harem cages do not maintain pedigree unless all females but one are removed from the male, and the others separated to allow identification of the dam for each pup. Rotation mating is hard to utilize when the females are left together as you have to wait until the additional litters are weaned.

General notes:

In some cases, multiple adults with multiple litters in a cage is permitted: for example, poor breeder strains (stock or genetically modified) where the average litter size per female is less than the average litter size of unaffected strains (i.e. the overall number of pups from two females is equal to, or less than, an average litter size from an unaffected strain); fragile strains, where the pups benefit from the additional social enrichment/nurturing of additional adults.

Alternate breeding strategies, such as adding a companion/auntie/nanny female (usually a surplus female, or retired breeder female) to singly housed pregnant females, may also be employed.

5. BREEDER AGES

Breeding animals should be mated and retired at appropriate ages for the species and strain. The maximum age for breeders must not exceed the natural reproductive lifespan for the species and strain (See Policy 013 statement #4). Generally in rodents, breeders are set up between 6 -12 weeks of age, and retired between 6 -12 months of age. Strain characteristics, including breeding performance, phenotypes, or behaviour, may influence the age at which breeders are set up and retired. The maximum age(s) must be described in the ACC breeding protocol.

6. WEANING

Age at weaning will vary by study, species and strain. Most rodent species should be weaned between 18 and 28 days of age. Weanlings should be separated by gender and housed at the

appropriate density for the species, strain, body weight, cage type (static, vented), and cage size.

Early and late weaning (specified by species and strain) must be described in the ACC breeding protocol.

Some rodents (generally mice) which would benefit from a later weaning age (e.g. up to 28 days) include: runted pups, genetically modified animals that have phenotypes making them weak or small, stock/inbred strains known to have small litter sizes or small pups (e.g. C57BL/6 mouse strains), and genetically modified strains maintained on one of these strain backgrounds, etc.

In cases of late weaning, attention must be paid to ensure two litters are not born into a cage with only one dam. This can be done by removal of the male prior to the previous litter being born to avoid post-partum estrous. See also overcrowding below.

Family/group weaning (where females and males are weaned together) may be done in cases where the pups are small, but cannot be left with their parents for later weaning. These cages must be clearly marked to indicate both sexes are together, and give a clear date by which they will be separated. Generally this is done 5-7 days later, prior to the onset of sexual maturity. If this is common practice for the strains, it must be outlined in the breeding protocol.

**Species specific notes: Male mice should not be recombined (regardless of age) as they are likely to fight, resulting in wounds and possibly requiring euthanasia). Female mice may generally be recombined at any age. However, it is not recommended to recombine female mice impregnated by different males, as this may induce the Bruce effect (pregnancy block, resulting in pregnancy termination).

Male and female rats may be recombined (generally at any age) but should be monitored for fighting.

Table 1: summary of mice/rat breeding/weaning information.

	Sexual Maturity	Reproductive Lifespan	Gestation	Litter Size	Weaning Age
Mice	5-8 weeks	~8-10 mo	18-21 days	2-12+	18-28 days Ave.21 days
Rats	8-10 weeks	~8-10 mo	21-23 days	4-20+	20-25 days Ave. 21 days

7. CAGE DENSITY

Cage sizes and housing density should permit rodents to perform behaviours important to their welfare during the reproductive and development stages and prevent overcrowding.

For breeders, the minimum floor area should meet the current standards of the CCAC, and take

into account the size of the litter(s) born, how big the pups are at wean, and the overall fecundity of the strain.

In cases where animals are group housed (e.g. dedicated trios, harems), larger cages should be considered to ensure these standards are met (see overcrowding below).

Wean aged animals should be separated by sex into appropriate sized social groups (see weaning above).

Cages are considered overcrowded if:

- A new litter is born before the older litter from the same female has been weaned (a "double litter").
- Females from trio or harem cages are not separated prior to any one female giving birth and if still housed with the male. Exceptions must be justified and approved in the ACC breeding protocol.
- Females do not have sufficient space to be away from the litter. For example, rats with healthy litters spend a maximum of 1 hour with pups at a time.
- A litter remains with the female beyond 21 days of age, as most litters can be weaned between 19-21 days (mice) and 20-25 days (rats) (with exceptions noted above).

8. ENVIRONMENT

Temperature, humidity and light cycle must all be controlled and maintained in a species specific manner, to which they can adapt with minimal stress and physiologic alteration. Exposure to wide temperature and humidity fluctuations or extremes may result in behavioral, physiologic, and morphologic changes, which might negatively affect animal well-being and research performance as well as outcomes of research protocols. These effects can be multigenerational. Maintaining scent free facilities is also strongly recommended.

Temperature: Most rodent facilities are maintained below the normal thermoneutral zone of rodents, (a range of 20-26C for both mice and rats), to allow for human comfort at ambient temperatures.

Variations in environmental temperature have been shown to affect reproductive performance. It is recommended to keep gestating and lactating rat dams, and pups up to 3-4 weeks old, at the higher end of the recommended temperature range (24-26C), as they have reduced thermoregulatory ability.

The addition of extra nesting material (see below) can help to achieve appropriate thermoneutrality for the rodents without adverse effect on the humans working with them.

Humidity: Most animals do well at 40 to 60% relative humidity, but not less than 35% or greater than 70%. The relative humidity should be kept consistent (± 5%). Low relative humidity, especially in combination with temperature extremes, may lead to ringtail, a condition involving ischemic necrosis of the tail and sometimes toes.

Light: The light-dark cycle has significant effects on mouse reproduction. Generally, the

light/dark cycle is 12/12, but in some cases extending the light period (14/10) can improve reproductive success.

For example, the estrus cycle is very sensitive to constant light. Constant exposure to light for only 3 days may cause persistent estrus, hyperestrogenism, polycystic ovaries, and endometrial hypertrophy or metaplasia.

Light intensity also affects reproduction with the greatest weight gains of pups seen at lower light levels (Lux). Strong light appears to decrease the survivability of the pups by adversely affecting lactation in the dam, and inducing emotional stress or hormonal imbalances.

Scents: No scents is good sense. Rodents have limited vision, and rely on their sense of smell (i.e. scent marking) to communicate. Scents also play an invisible role in priming reproductive physiology and development. Exposure to odours/scents (perfumes/colognes, scented lotion, shampoo, soaps, etc.) can dramatically alter their behavior and affect reproductive success. Exposure to male pheromones has also been shown to cause stress and related analgesia in rodents.

9. NESTING MATERIAL

All cages must be given ample nesting materials appropriate to the species, strain, size and number of animals, and the caging system in use. Nesting material provides comfort, helps regulate temperature and light levels, and provides a place to hide and retreat from cage mates or threatening stimuli. Nesting material that needs to be shredded, also provides enrichment and novelty to the cages. Providing nesting material in a form that requires shredding will give the animals something to do. Nesting material must be provided to pregnant females and females with litters.

Nesting material is usually a combination of loose materials that can be used to form an appropriately sized nest. Examples include “crinkle” paper (e.g. Enviro-dri®), cotton nestlet squares (e.g. ALPHA-Nest®), paper towel/tissue, etc.

10. IDENTIFICATION OF RODENTS

In most cases, it is important to be able to identify individual animals and the method should ensure a permanent, indisputable identification of an individual, without negatively impacting animal welfare. Animals used in breeding colonies or moved from breeding colonies into research colonies should have unique IDs which allow them to be tracked easily. This is vital in cases of disease outbreak or unexpected phenotypes, where tracing back to the original cage can prevent an entire colony from having to be euthanized.

Please see the CCAC Brochure on Rodent Identification and UBC ACC Policy 019 for all acceptable methods of rodent identification.

11. RECORD KEEPING

A. Cage records: Minimum requirements for information:

1. Breeding cages: PI name, ACC protocol #, date of mating set-up, dam/sire information (strain, date of birth, IDs, generation (for certain breeding schemes)), predicted/actual parturition and weaning dates, and litter information (date litter(s) born, number born and/or weaned, sex of weaned pups). See also UBC ACC Guidelines on Cage Card Requirements and Sample Cards.
2. Post-weaning cages: PI, ACC protocol #, strain, sex, date born, date weaned, number of animals per cage, IDs (e.g. genetically modified animals, or animals moving onto a research protocol), and, if genetically modified, whether positive for the transgene or mutation.

Additional recommended information includes intended use (breeding, experiment, and protocol #), coat colour, or other unique identifiers.

B. Lab records: To track the number of offspring produced as the number of animals used/produced must be kept and reported annually to the ACC. Lab records should include the reason and date any offspring were euthanized or died, whether mice were recycled into the breeding colony, or transferred to a research protocol or to a different investigator.

Use of electronic colony management based systems (e.g. Mosaic Vivarium, or similar) are strongly recommended.

12. STRAIN NOMENCLATURE

Strain nomenclature for rats and mice is standardized across the international research community. While complex, it is necessary. Proper strain nomenclature allows you to specify: background strain (e.g. pure, hybrid, mixed, unknown), strain development/origins, and any mutations present (the type (knock-out, knock-in, targeted, etc.), the gene affected, and the transgenic allele). This information can then be used to identify the strain and appropriate controls.

Nomenclature is especially important during the generation of new strains. When two genetically distinct strains are intercrossed (whether they are stock or transgenic/GE), this new strain must be identified and described in the protocol. See Policy 013 statement #2.

Where two strains are being intercrossed for the first time, and the resultant phenotype is unknown, or may affect their health and welfare, specific colony monitoring must be implemented (see Policy 013 statement #10). Note this strain must be described in the breeding protocol, and may necessitate a research protocol wherein the new strain is further described.

13. ACCLIMATION AND QUARANTINE

Acclimation of the animals following transport and exposure to a new housing environment is preferred as it results in a less stressed and healthier animal prior to breeding. This acclimation period is often completed while animals are in quarantine. The requirement for quarantine and health testing of animals being imported into a facility, especially from non-commercial sources, is set out by each facility, and should be discussed in advance of any animals being

ordered.

14. REFERENCES

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http://www.ccac.ca/en_/training/niaut/vivaria/animal_care/brochure

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- Policy 2: Transport of Research Animals Between Facilities at UBC
- Policy 3: Transport from UBC
- Policy 19: Acceptable Methods of Rodent Identification
- UBC ACC Guidelines on Cage Card Requirements and Sample Cards

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