



An Overview on the Management of Laboratory Mouse Colonies

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【Abstract】 Two lectures covering the management of laboratory mouse colonies were presented in November at the laboratory animal medicine technical training seminars sponsored by the Chinese Association for Laboratory Animal Sciences in Xi'an. Topics covered include troubleshooting mouse reproductive performance and managing genetically engineered colonies. The purpose of this article is to highlight the materials covered and to offer references for further reading. It is advised that references cited in this article be reviewed for a more comprehensive coverage on the topics presented. Please also note that the principles for breeding practice and genetic management of outbred mice were not covered, but the author may be contacted for a list of suggested reading materials.

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实验小鼠种群管理

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【摘要】 2009 年 11 月份在西安举行由中国实验动物学会发起的实验动物医药技术培训研讨会上, 本人做了关于实验小鼠种群管理的演讲。其主题涵盖了小鼠繁殖性能的维护和基因工程种群的管理。本篇文章的目的在于强调其所涵盖的材料并为进一步研究提供参考。如若对本文所讨论的内容希望有更深刻的理解, 这篇文章所引用的参考资料值得进一步的阅读。另外本文未涉及远交系小鼠的繁殖培育和基因管理, 但如有需要可以联系本文作者, 可推荐需阅读的材料清单。

【关键词】 小鼠; 种群繁育; 管理; 产量

1 Reproductive Performance of Mice

Rodent reproduction is a complex process subject to biological and environmental influences. There are many textbooks that provide information on the general biology and reproductive physiology of the laboratory mice. Although variability can exist between different mouse strains and stocks, and sometimes unpredictable in genetically modified mouse models, such information can and should serve as a baseline with which to

establish one's breeding management practice. Any mouse user should have a good understanding of basic rodent biology and physiology, as well as knowledge regarding rodent genetics in order to successfully manage the colony while meeting production and scientific goals. Two suggested references include an article by Murray and Parker^[1], which contained examples and tips for troubleshooting reproductive performances of mice, and the book chapter by Pritchett and Taft on the reproductive biology of the

laboratory mouse, which provided comprehensive coverage on the topic^[1].

Breeding systems fall under two general categories. First is the permanently mated groups, which include monogamous and harem breeding. Second, the temporary mated groups, which include polygamous and timed-matings, where the females are separated from the males at some time post mating. Advantages and disadvantages associated with each system are discussed by Murray and Parker^[1]. To maximize the productivity of female mice, they are best kept in permanently mated groups to take advantage of the post-partum estrus. Monogamous breeding cages will result in the most number of pups born per female over her reproductive lifespan. On the other hand, having multiple females in the same cage (i. e. harem breeding) will result in the most number of pups born per breeder cage, although inadvertently individual productivity will decrease. To maximize the productivity by a single male mouse, it may be best to rotate different receptive females to the stud cage on a weekly basis.

Mating scheme refers to breeder set-up based on genetic background and zygosity of the breeders; for example, mating of a heterozygous male to a homozygous female. When breeding genetically modified rodents, the mating scheme will depend on the phenotype of the model, the production goals, and in some cases, limited by the availability of the breeders. To minimize breeding errors, mouse users should be required to have a good understanding of basic principles of genetics.

2 Maintenance of Breeding Records

Keeping organized breeding records is an essential part of production colony management. It allows one to generate reproductive performance data that are reflective of one's own production colony, as well as help one establish realistic expectations and troubleshoot production.

Basic breeding information can easily be tracked at cage side. For example, on the cage card one can list the identification and date of birth of the breeders, date of breeding unit set-up, age of the breeders at first litter, the dates of all litters born, number of pups born

and weaned in each litter, sex distribution within each litter, and expected retirement age/date of the breeders. The retained information can further be used to assess the reproductive performance of a particular colony. For example, the frequency with which each mating pair or unit will produce offspring (e. g. productive mating frequency), the age at which the female will first become pregnant, typical litter size, interval between litters, cumulative number of litters and/or offspring, and the average number of litters that a single female can produce over her life time.

Suggested reading on systems for keeping breeding colony records include the chapter by W. White on management and design of breeding facilities^[1], the chapter by Festing and Peters on animal production and breeding methods^[1], and text by L. Silver on mouse genetics^[6].

3 Breeder Selection and Replacement

Keep in mind that a mouse colony consists of three functional categories: breeders, future breeders, and stock. Active breeders produce stock animals that are used on studies, but a certain number of mice need to be set aside as future breeders to replace a portion of current breeding pool as their reproductive performance declines. In general, select animals that exhibit the normal characteristics for that strain or stock as future breeders. When maintaining inbred colonies, choose sibling pairs from parents that exhibit the standard phenotype reported. Make sure a breeder rotation schedule is established in order to sustain the colony size while meeting short and long term production goals. The breeder rotation schedule will depend on the effective reproductive lifespan of the animal model. For example, if the effective reproductive lifespan of your breeder is roughly 5 months, then it is recommended that 20% of the existing older breeders be retired and replaced every month. If the effective reproductive lifespan is 10 months, then 10% of the breeding pool should be rotated off every month^[6].

Most mice should be producing a litter every 3 to 4 weeks, with a visible decrease in reproductive performance as they age. Mating cages that are exhibiting poor reproductive performance and not meeting expectations should be investigated, and

possibly replaced. For example, mating cages that do not produce any litters after 2 to 3 months after set-up, lack of new litters produced after 2 months, or consecutive loss of 3 litters due to inability for a cage to raise or wean pups. Mice that exhibit health issues or have anatomical deformities are not likely to be effective breeders. This includes certain disease models that are genetically predisposed to shortened lifespan or sub-optimal parenting abilities, and timely replacement of breeders or setting up alternative breeding schemes should be used.

Through periodic review of production data, a good colony manager should be able to further characterize the reproductive performance and lifespan of a particular model, as well as effectively troubleshoot production problems and implement appropriate corrective measures.

4 Troubleshooting Production

Many factors related to the animals and their environments are known to influence the reproductive performance of mice, and should be considered when troubleshooting production problems. For a more detailed list and in depth review of these factors, please see the chapter by Pritchett and Taft^[1]. Historic data have shown that the reproductive performance of mice varied by strains and stocks, and these data can be found in various texts^[6, 51]. Specific strain characteristics or mutations can also impact the parenting ability of mice. Biological problems that influence production can include a combination of age, body condition, health, strain type, and genetic mutations. Environmental parameters known to effect production include caging system, bedding, diet, temperature humidity, light cycle, noise and vibration^[2, 7-9].

As increase in animal exchange occur between institutions, rodent colony managers must take into considerations that new mice are exposed to unknown microorganisms in the environment that may subclinically or clinically impact their health and reproductive performance. Additional challenges include sharing of incomplete genetics and breeding history, and the need to establish a colony based on a few individual animals. Finally, creation of novel

animal models through genetic manipulation may sometimes render the phenotypic expression and reproductive performance unpredictable. These scenarios can present as hidden problems that interfere with effective management of a production colony.

When realistic production expectations are not being met, one should take a systemic approach to identify the problems(s). Scrutinize the animals, their environment, and their genetics. Determine if the problem is the result of mice not mating (e. g. lack of copulatory plug), if the mice are not getting pregnant, if pregnancy is noted, but no pups are born, if pups are born but are not raised, or dies before weaning. Or perhaps production level is not meeting the expected number or genotype distribution, model phenotype and performance? If there is a problem perpetuating valuable animals, assisted reproductive technologies (ART) are available for rescue purpose^[60].

If the problem is that mice aren't breeding, examine the breeders within the cage to rule out any age, anatomical, or health related issues that might have interfered with successful mating. One may change one or more environmental parameters (e. g. light cycle, diet type, supplement provision, environmental enrichment, cage/rack location, etc.) to see if there is an improvement. Alternatively, set up the problematic breeders with different partners in new cages. One may introduce young, wild-type animals from vendors into the breeding pool. For older females potentially experiencing prolonged anestrus, it has been suggested that low dose gonadotropin injections may be used in an attempt to "jump start" the estrous cycle. Typically a strain dependent dose that is half the usual dose for prepubescent animals is recommended, but effectiveness is not always guaranteed. Alternatively, ART such as ovarian transplantation, embryo transfer, *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI) are commercially available.

If the problem is that mice are mating as evident by the presence of a copulatory plug, but does not result in pregnancy, then one should replace the male breeder, or perform IVF after assessing his sperm quality and quantity. Fertilized embryos are then implanted into surrogate females to perpetuate a line. If

the females do show signs of pregnancy, but do not litter as expected, consider potential causes of abortion or fetal resorption. The possibility of nutritional deficiencies or toxicity, subclinical disease state, genetic manipulation, and environmental stressors should be investigated.

If the problem involves post-natal lethality, check for milk-spots in newborn pups to ensure there is no lactation problems with the dam, and that the pups have normal suckling ability and have been nursing. Diagnostic workup may be necessary to rule out infectious agents of colony health concern, such as rotavirus or mouse hepatitis virus, which are known to cause morbidity and mortality in susceptible preweanlings. If dealing with novel genetically engineered mouse models, it will be important to perform genetic testing on the pups to rule out the possibility of a detrimental phenotype and adjust breeding scheme accordingly. If necessary, consider cross-fostering the pups to salvage valuable litters. Where appropriate, as an alternative to cross-fostering, ovarian transplantation may be used to rescue a line when the colony has been decimated down to unproductive females. Additional tips on cross-fostering and ovarian transplantation may be found in the chapter by Pritchett and Taft^[1].

In a typical closed inbred colony, the production level will drop as result of inbreeding depression. If all of a sudden a robust production is observed, then one should suspect hybrid vigor and test the colony for potential mismating or genetic contamination. If there is an unexpected distribution of offspring genotypes, for example, lack of homozygous pups from mating parents heterozygous for a mutation, it is good practice to retest the parents to confirm their genotype, then consider *in utero* or post-natal mortality. To confirm suspicion, one could perform timed-mating to check the genotype of embryos or fetus at different gestation stages.

If one is following the production planning calculations, but is still unable to meet production goals, ART may be used to generate a large number of animals within a short period of time. Depending on the availability of the breeders, one could superovulate mice with exogenous hormones, then either collect oocytes for IVF or time-mate the animals to collect

stage specific embryos for embryo transfer. For superovulation and IVF protocols, a mouse embryologist should be consulted. Dosage and success of superovulation will be dependent on animal strain, age, and weight.

5 Importance of Genetic Background and Nomenclature of Mice

One cannot stress enough the importance of genetic background and use of proper nomenclature when working with mouse models. The degree of gene expression, or animal phenotype, can vary depending on the genetic background of the model. Many commonly used inbred strains exhibit strain specific pathologies, or “background lesions,” that can confound the result of genetic manipulation. Further inbred strain characteristics may be found under Mouse Genome informatics website hosted by The Jackson Laboratory (www.informatics.jax.org/external/festing/mouse/STRAINS.shtml). Finally, the behavior of mice can vary by vendors and production sites, by strains and substrains, and is subject to external influence (i. e. environmental conditions, husbandry practice) under which the animals were raised. Therefore, whenever possible, pay close attention to factors that have the potential to alter the performance of the animal model.

The proper mouse nomenclature serves as a unique identifier of mice, convey important technology, line, and producer information. In scientific publications, proper mouse nomenclature should be used in order to ensure reproducibility of that particular study. The use of proper nomenclature is also pertinent for animal purchasing agents to ensure the correct animal models are ordered and used for studies. Knowing the proper nomenclature of the mouse model will allow for more effective management of breeding records and pedigree information. A thorough review of mouse nomenclature may be found in various reference texts^[6, 11], while the current guidelines are hosted on The Jackson laboratory website (www.informatics.jax.org/nomen).

6 Maintaining Genetic Integrity

The utilization of genetically defined mice and their

proper controls is essential to the validity of an experiment. In any population, the prevalence of various genotype/phenotype is constantly changing, and genetic divergence can occur due to spontaneous mutations, natural selection (though less likely in our controlled laboratory setting), unconscious selection, genetic drift, and population migration. If two populations are separated geographically, their genotype and phenotype will assort independently and likely diverge from one another. Certain phenotype or genotype may become fixed or eliminated from one population or another, and if different populations are assayed over time, one will see that the distribution of genotype/phenotype varied in an unpredictable fashion.

In the management of mouse colonies, sources of genetic variability may result from human error (e. g. genetic contamination, incomplete inbreeding resulting in residual heterozygosity), intentional genetic manipulation, or the unmonitored accumulation and fixation of spontaneous mutations that occur over time (i. e. genetic drift). This often accounts for the variation seen over time in research results, despite the control of all external parameters. Managers of mouse breeding colonies, especially those working in institutions that maintain small or closed colonies, should be aware of this and implement measures that will control potential sources of genetic variability.

In addition to becoming familiar with specific strain characteristics, training of mouse users should include basic rodent genetics, proper record keeping, and recognition of deviant phenotypes. Biological control measures may include capturing and euthanizing all escaped mice, cryopreservation, and establishment of foundation colonies. Strains of the same coat color should be separately housed whenever possible, or else use color coded cage cards to distinguish between populations.

When appropriate, testing should be performed to monitor the genetic integrity of the colony. Genetic testing can include the zygosity testing for genes(s) of interest (i. e. genotyping) and background testing, which identifies genetic background composition as related to a particular mouse strain. Prior to expansion of a colony using only a few animals received from a collaborator, it is always a good idea to perform initial

characterization with regards to the genotype and/or genetic background of the mice to make sure the animals are what you expect, as well we determine which substrain they are in case it is necessary to acquire wild-type mice from a vendor. Discussions related to genetic monitoring, including various techniques used for genetic testing in mice, may be found in many published references^[2-15].

7 Meeting Production Expectations

In order to meet production expectations, it is important to understand the purpose of the breeding colony. For example, whether the purpose is to supply animals involved in embryo transfer projects, to create and expand a specific founder/chimera line, to generate cohorts of animals for scientific experiments, or to simply maintain backup colonies when large scale production is occurring elsewhere. The goals for any breeding colony also needs to be clearly defined, whether there is a specific number of animals required over a given period of time, and what the required characteristics are (e. g. sex, age range, genotype). Furthermore, in order to set realistic expectations for any production colony, information regarding the background strain, its production indices, reproductive lifespan, maternal attributes, availability of breeders, mating systems and breed schemes, model phenotype and health status must all be taken into consideration.

Historic information regarding the reproductive characteristics of different basic mouse strains may be found in texts by L. Silver and W. White^[6,51]. Tables 1 and 2 are adapted from published texts for the purpose of demonstrating the process for production planning calculations. Data relevant to one's own mouse colony should ideally be derived from breeding record data maintained within your own institution, since data will vary due to the combined interaction of animal reproductive ability, production systems, and the breeding environment.

8 Production Planning

To plan production, one needs to know the number and the characteristics of animals needed, as well as the productive mating frequency for the strain. For example, if breeding BALB/cJ mice, or

genetically manipulated mice on BALB/cJ background, where the anticipated productive mating frequency is 47% (table 1), one may expect that after setting 10 females up to breed, only 4 to 5 mice will produce litters. Therefore, if a study requires a minimum of 10 pregnant females, it may be necessary to set up twice as many animals to breed. Then, based on the actual production outcome, the breeding population may be adjusted accordingly.

Production index (PI), on the other hand, refers to the number of animals weaned per female in the breeding colony per week (i. e. $PI = \text{pups weaned} / \text{female/week}$). Mice of C57BL/6 background has a PI of 0.5 (table 2). Therefore, in a scenario requiring continuous production, one would expect to wean 0.5 pups each week per breeder female. To calculate the PI for an internal colony, simply count the number of weaned animals, and not just those born, over a number of weeks, and all of the females used in production, not just those with litters. The longer span of breeding data collected, the more accurate the calculated number will reflect the colony status.

During production planning exercises, the mathematical equation used can be listed as follows: # females $\times$ PI = # pups weaned/week. If productive mating frequency information is available, it should be

taken into consideration in the arithmetic in anticipation that some mice set up to breed may be unable to mate, and are considered infertile^[1].

As an example, if a researcher needs 20 homozygous (HO) mutant males on a C3H background every week for 6 weeks, and only heterozygous (HE) mice are available for breeding, what is the minimum size of the breeding colony required? One should understand that only 25% of the animals produced from a HE $\times$ HE breeding scheme will be of the desired genotype. Assuming equal distribution of males to females in a litter, only 12.5% (or 1/8) of the offspring produced will be useful. Therefore, each week $20 \times 8 = 160$ pups will need to be produced in order to meet production goal. Using the equation # females $\times$ PI = # pups weaned/week, where # pups is 160 and PI is 0.8 for C3H mice, then # females = $160 / 0.8 = 200$. In addition, knowing that productive mating frequency is 86% for C3H/J substrain (table 1), it may be necessary to increase the number breeder females to 233 (e. g. 200 divided by 0.86) in anticipation that roughly 14% of the animals set up may not breed as expected. This simple arithmetic provides a rough estimate for the number of breeding females required to sustain the production goal of 20 homozygote males each week for 6 weeks.

Tab.1 Reproductive characteristics of common inbred strains^[1]

Strain	Productive Matings	Weeks at First Mating	Litter Size	Number of Litters	Relative Fecundity
BALB/cJ	47%	8.0	5.2	3.8	9.3
C3H/HeJ	86%	6.7	5.7	2.9	14.2
C57BL/6J	84%	6.8	7.0	4.0	23.5
DBA/2J	75%	7.4	5.4	3.9	15.8
FVB/N	> 90%	—	9.5	4.8	41.0

Note: Matings are considered “productive” if any offspring at all are born. “Weeks at first mating” refers to the age at which the first “productive” mating occurred. “Relative fecundity” is a value that measures the overall fecundity of the strain, and is obtained by multiplying (productive mating frequency) $\times$ (litter size) $\times$ (number of litters).

Tab.2 Typical production indices for common inbred strains^[1]

Strain ^a	Production Index
BALB/c	0.8
C57BL/6	0.5
C3H	0.8
DBA/2	0.35
FVB	0.8

Note: ^aBreeder information is unknown. Production index (PI) refers to the number of animals weaned per female in the breeding colony per week.

Ultimately, there is no guarantee that the animals

will breed when expected, produce the number of offspring expected, or produce animals of the expected genotype or sex. Therefore, more animals may need to be included, especially if one is setting aside animals as future breeders in addition to producing study animals. For more information and examples of production planning, please refer to the book chapter by W. White,^[1] appendix B in the Guide for the Care and Use of Mammals in Neuroscience and Behavioral

Research,^[6] or the resource manual available from The Jackson Laboratory^[6].

9 Disaster Planning

When managing rodent breeding colonies, especially valuable mouse lines, it is important to plan against disasters, including natural disasters that can cause one to lose an entire colony, or potential genetic or microbiological contamination within a colony. One practice is to split colonies and manage animals in different rooms or different facilities. Another practice is to cryopreserve the embryos or sperm of valuable lines. The choice to cryopreserve embryos vs. sperm will be dependent on one's ability to reconstitute the line in the future, the genotype requirements post-reconstitution, and the availability of donor animals. Compared to splitting colonies, cryopreservation has additional advantages. For example, it is a viable, cost-effective long-term storage solution that prevents the loss of a line, while allowing other active colonies to occupy vivarium space. This practice not only slows down genetic drift within a colony, but also rederives the colony when reconstituted, sharing the health profile of the recipient animals. Finally, frozen germplasms may be shipped to collaborators with less cost and more ease as compared to shipping live animals.

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