GV-SOLAS
Gesellschaft für Versuchstierkunde

# Specialist information

## from the Committee for Genetics and Laboratory Animal Breeding

# Reducing surplus experimental animal generation

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## Description of the situation

### Background

Biomedical research today is inconceivable without the use of genetically modified animals, especially mice. On the one hand, mice are well suited as experimental animals due to their small size and short reproduction times, and on the other hand, techniques for the targeted modification of the genome in mice have been established for several decades. In the meantime, these techniques have also been applied to many other animal species and are now at an advanced stage of development. Today, there are thousands of animal models that carry well-defined genetic alterations and are selectively bred in laboratory animal facilities. **This short review is intended to deal with those animals that are inevitably born due to genetics and the breeding procedures based on it but cannot be used for the actual intended scientific purpose. Furthermore, this publication points out strategies and approaches how to minimize the number and emergence of these animals as much as possible. Finally, possibilities will be discussed on how surplus animals can be used for other purposes.** Since genetically modified animals are usually produced by genetic engineering techniques, animals generated in this way are subject to the European regulations on genetically modified animals as well as national genetic engineering acts and by-laws.

### Replacement, Reduction, and Refinement (3R)

It is the declared aim of the GV-SOLAS to promote the humane use of laboratory animals. The focus of these considerations is on the so-called 3Rs: Replacement, Reduction and Refinement. Genetic modifications in laboratory mice are often used to model diseases in humans, but also in farm and companion animals. It is therefore obvious that these genetic alterations may also be associated with animal distress. According to the 3Rs mentioned above, the goal of researchers must be both to reduce any potential burden caused by the genetic modification (refinement) and to keep the number of animals used in animal experiments as low as possible (reduction).

## Minimizing the number of laboratory mice bred

### Causes for the emergence of surplus animals

For biological reasons during the breeding of genetically modified mice it is in many cases impossible to generate exclusively animals with only such traits that can then be used in the planned experiments (see Fig.1). Inevitably, animals are also born that do not carry the required traits. These can then only be partially used in animal experiments (e.g., as control animals) or for further breeding.

To keep the number of surplus animals in experimental animal breeding as low as possible, it is necessary to establish an optimized breeding strategy that meets the needs of the researchers. Furthermore, the animals from the breeding programs, most of which do not carry a harmful genotype, should be put to good use. Below we discuss several possibilities to achieve this.

## Optimization of breedings

The principles of inheritance of genetic traits, as discovered by Gregor Mendel, form the basis of any breeding setup in laboratory animal husbandries. It follows from these inheritance rules alone that a considerable proportion of animals in a breeding program will not have the experimentally required genotype (see Fig. 1). **Despite these immutable biological laws, any institution can develop strategies to avoid unnecessary breeding, as well as efficiently perform the necessary propagation by use of optimized breeding schemes.** Through precise planning and calculation, a breeding strategy can be developed that involves the smallest possible number of animals without running the risk of falling below the group size statistically required for an experiment. Because of the possible variables, no standardized procedure can be given here, but there is guidance (1, 2, 3) and software (4) available that allow optimized breeding planning.

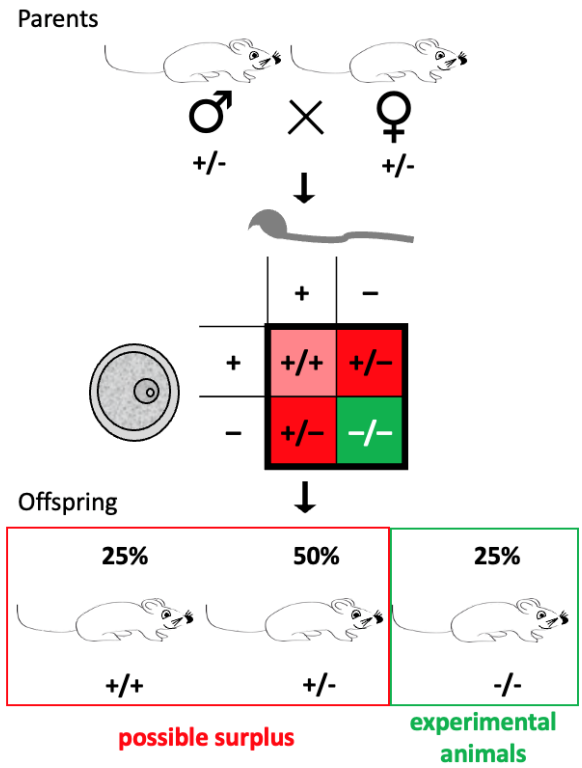


**Fig. 1 Mendel Genetics** All mammals carry each chromosome twice. However, the genes on two sister chromosomes can have different variants. Here we show the offspring of the breeding of two parent animals which carry two different variants of the same gene (denoted + and -). Thus, the sperm and egg cells can each carry the + or - variant. If now, as shown in the Punnett square* in the middle, the different egg and sperm cells are combined, this will form the genotypes of the next generation. The square also shows how frequently each genotype occurs. For example, if researchers need only -/- animals for their analyses, they can only use 25% of the animals as experimental animals while 75% cannot be used in the experiment. This is the result of natural breeding laws and cannot be influenced by the researchers.

* after the British geneticist Reginald C. Punnett

## Preventing unnecessary breeding through cryopreservation

Genetically modified lines that are not to be used immediately for experiments should be frozen (cryopreserved) in the form of embryos or sperm. Cryopreservation has many advantages, such as reducing the number of animals in breeding, costs, prevention of spontaneous mutations/genetic drift, avoidance of animal transports, and maintenance of animal health. Yet it is also sometimes criticized because the process itself requires animals and revitalization of

a conserved line involves the use e.g., surrogate mothers which have to undergo harmful procedures. Despite these criticisms, cryopreservation is a very essential technique for securing mutant models, which entails a considerable reduction in breeding. It must be decided in each individual case whether the cryopreservation of a line appears reasonable or whether it is necessary to continue its breeding.

**Tissue-specific and induced gene alterations.**

It has been possible for some time **to induce genomic changes in a time- and/or tissue-specific manner.** The most commonly used method requires two genetic components that are brought together by crossing two specific lines. This has the advantage that the individual lines can be maintained in breeding colonies without any harmful phenotype as a result of the genetic modification (refinement). However, the disadvantage is that breeding double-mutant animals also results in animals that cannot be used for the planned experiment due to the rules of heredity. This runs counter to the idea of reducing the number of experimental animals. Consequently, the best possible approach must be decided on a case-by-case basis. Siblings from such breedings can serve as control groups for the intended experiments.

**Sharing with third parties**

In many institutions, **platforms have been created to enable the exchange of animals or organs for further scientific use.** However, the terms and conditions of various breeding service providers as well as contracts (Material Transfer Agreements, MTAs) with other institutions often restrict the transfer of animals to third parties to protect intellectual or commercial property. Animal testing facilities may therefore only distribute their own lines without restriction or require permission from the licensor. **Organs from animals can in principle be transferred by a facility to outside parties. Appropriate platforms, such as preclinical biobanks, are useful for this purpose, too. Live animals can also be transferred externally, e.g., into private hands,** if "rehoming programs" are established in accordance with the applicable legal provisions (e.g., genetic engineering legislation). However, in contrast to dogs and cats, rodents are extremely difficult to rehome, especially in large numbers. Also, genetically modified animals are generally excluded from placement into private hands.

**Use in education, training, and continuing education**

Animals without harmful mutations are very well suited for use for training purposes of staff and experimenters in individual training or experimental animal science courses. From an ethical point of view and according to the principle of harm minimization, only a few manipulations should be carried out on the same animal and thus preference should be given to lower stress on the individual animal (refinement) over minimization of the total number of animals with individually higher stress (reduction). Competent authorities are generally in favour of this approach. However, the weighing must be done on a case-by-case basis.

### Use as feed animals

Zoos, falconries, reptile, and birds of prey sanctuaries, etc. have a large demand for animals to feed the animals they keep. This need is partly met with rodents that are specially bred as feed animals. Facilities for breeding laboratory animals maintain a very high standard of animal welfare and hygiene. These high standards are usually not achieved by breeders of feed animals. To hand over surplus laboratory animals as feed animals, the relevant legal provisions must be complied with (registration according to Art. 23 VO EG 1069/2009).

This is possible with only moderate bureaucratic effort for wild-type animals and for mutant animals that have not been generated by genetic engineering measures (spontaneous mutants). Primary contacts are usually the local veterinary offices.

However, this possibility does not include dead and inactivated genetically modified animals. They would be considered genetically modified feed as defined by EU Regulation 1829/2003. To be able to use genetically modified animals as feed animals, an individual approval of each animal line as feed according to this regulation is required, which makes the distribution of such animals almost impossible. To our knowledge, there is no precedent to date for approval of genetically modified mice or rats as feed.

In terms of animal welfare, the aim should be to allow the feeding of dead genetically modified animals, as it is contrary to the concerns of animal welfare to dispose of dead laboratory animals of the highest quality unused on the one hand, while on the other hand animals are bred and killed under inferior standards specifically for feeding.

## Conclusion

Because of mammalian genetics, animals are born in laboratory animal facilities that do not carry the traits needed for research. The number of these animals that cannot be used in experiments can be minimized, but it is impossible to completely avoid producing such animals. If no experiments are planned in the longer term, genetically modified lines can be cryopreserved. The use of inducible and tissue-specific gene modifications helps to avoid harmful phenotypes, but also regularly results in higher numbers of surplus animals. Giving animals to other research groups or to private hands or using them in laboratory animal science education are ways to make good use of animals that cannot be used in own research projects. The alternative use of genetically modified animals, which cannot be used for scientific purposes because of their genotype, as feed animals is practically limited to wild-type animals or to such genetically modified animals that have not been created by genetic engineering methods (spontaneous mutants). Passing on genetically modified animals as feed is virtually impossible for regulatory reasons. There is an urgent need for legislative action in this regard at EU level.

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