Breeding, an essential but often overlooked part of animal experimentation

Isabelle Grandjean directs the animal facility at the Agora research center. Pursuing a balance between scientific progress and respect for animals, she works closely with researchers to optimize their breeding strategies and thereby reduce animal numbers.

In her office, Isabelle Grandjean, head of the Agora animal facility (Agora In Vivo Center, AIVC) juggles between phone calls and her cup of coffee, always ready to greet her visitors with a warm smile. "I often hear: 'Do you have a moment for me? This will only take five minutes!' I know it's going to take more than that, but I don't care. I always find time for a chat."

After fourteen years directing the animal facilities at the Institut Curie in Paris, Isabelle Grandjean took over the AIVC's reins in December 2020, mandated by the Faculty of biology and medicine (FBM) of UNIL. The facility is home to animals belonging to research groups from the Lausanne University Hospital (CHUV), the University of Lausanne (UNIL), the Swiss Federal Institute of Technology of Lausanne (EPFL), the University Hospitals of Geneva (HUG) and the University of Geneva (UNIGE). Together, these teams form the Agora interinstitutional community of cancer research.

While being convinced that animal experimentation remains a critical tool of modern scientific and medical research, Isabelle Grandjean is no less sensitive to animal welfare, and for the past two years has been working with her team to raise only the number of animals strictly necessary for experiments. The **3R principle (Replacement, Reduction, Refinement)** being at the center of animal experimentation today, she is committed to making researchers aware that this principle must also be applied in the breeding of animals that are to be used in experiments. Interview.

Why does animal husbandry have a key role to play in the 3Rs?

In Switzerland, as in many other countries, only a fraction of the mice bred in our facilities are used for experiments. In 2022, this number was about 360'000, while the number of mice bred and/or imported was close to 1.03 million, so around 35 % of animals went into experimentation. Although these figures keep declining in recent years, it must be said that a considerable number of animals kept in animal houses are not directly used for research.

This is mainly due to an increasing use of genetically modified strains - for 2022, we are talking about 800'000 transgenic mice being bred in Switzerland. To date, no technology can guarantee that all mice being created will possess the genetic characteristics required for a specific experiment. However, as most of these lines are bred in our own facilities, it is our responsibility to ensure an ethical breeding management to comply with the 3R principle. By refining our coupling strategies, we aim to raise no more than the bare minimum of mice required for experimentation.

Why are animals bred and then not used in experiments?

There are four main reasons why some animals may not be used for experiments, even though they play an important role in the overall research process.

First, researchers may require an unequal number of male and female animals, leading to a sex surplus. This may be motivated by the field of research, for example regarding diseases that particularly affect women or men. If we take the example of prostate cancer or breast cancer,

only males or females will be used for experiments. For scientifically well-founded reasons, the exclusion of animals based on their sex can thus certainly be justified.

Second, the need to dispose of breeder animals when raising mice of interest for experimentation. These breeders are rarely used afterwards, due to their age or genetic characteristics.

Third, the obtained genotype in the case of breeding transgenic animals. In some projects, mice used for experiments may have up to four different genetic modifications. This leads to the creation of many animals which do not have the desired genetic characteristics and are therefore excluded from experiments. We cannot change the laws of Mendelian genetics, but we can apply them wisely to minimize the number of “unsuitable” animals.

Fourth, unpredictable demands linked to the rapid progression of science. All animal experiments must be approved beforehand by the authorities. The process of obtaining such a license is demanding and often takes several months. However, as experimental results are generated, certain scientific hypotheses may no longer be relevant and must be abandoned - or *vice versa*, preliminary results may pave the way for new hypotheses that require other types of experiments. This can lead to a temporary surplus of animals, as breeding programs cannot be adapted within the same time lapse.

How can breeding strategies be optimized to limit such overabundance?

Breeding is, after all, an essential part of experimentation, but one that is often overlooked by researchers when preparing their projects. Therefore, at Agora, the animal husbandry team works closely with researchers to develop carefully planned breeding strategies best suited to each research project. This collaboration has enabled us to obtain encouraging results in terms of both the number of animals used in experiments and the total number of animals kept in the facility, as illustrated by two examples.

In the first case, the research group had many genetically modified strains, each with its own characteristics and several combinations of mutations. To manage these complex lines efficiently, we analyzed each strain and each research question, and together established the best possible mating strategies. In just one year, we thereby managed to increase the number of genetically modified animals used in experiments by 32 %, without augmenting the total number of mice.

In the second case, we decreased the number of animals born in the facility by half, thanks to two key actions. First, we stopped breeding wild type strains* in-house, opting instead to buy them from specialist commercial suppliers. Acquiring animals from specialist breeders allows researchers to obtain exactly the right number of animals, of the right sex and age, at any given time. This approach is economically more advantageous, and without doubt ethically fairer. Second, we identified several genetically modified lines that were being maintained in anticipation of possible requests for further experiments as part of publication reviews. Instead of keeping these breedings going, we cryopreserved sperm or embryos. With today's technologies, it is possible to revive a cryopreserved line and obtain the mice of interest in almost the same time as maintaining the breeding in the animal house.

What resources are needed to make this collaboration between animal husbandry and research teams successful?

First and foremost, a common interest to cooperate is essential, all the while respecting each other's skills and expectations. This requires frequent meetings and a mutual understanding of

each other's work. To this end, we regularly organize sessions where researchers present their projects to the whole animal house team. It's a real opportunity to exchange ideas, to give meaning to the day-to-day work of the animal caretakers, and to encourage them to be even more involved in monitoring the animals undergoing experiments. *Vice versa*, I would like to organize practical sessions in the animal facility where the team welcomes researchers and shares some of their daily activities, such as changing cages, mating or weaning. I'm convinced that these encounters contribute to create "win-win" collaborations, where everyone is aware of each other's interests, in a way that's also good for the animals.

In addition, it is essential to have people with a solid background in genetics and animal breeding management. That's why I'm advocating for the creation of a breeding coordinator position within the Faculty, dedicated to supporting and advising the research teams in colony management, in close collaboration with the animal husbandry teams. In my vision, this person would support the role of our 3R coordinator, Dr Stéphanie Claudinot, specifically to educate and train facility users in colony breeding according to their individual needs. This would include, for example, knowledge of genetics, the standardization of strain nomenclature to facilitate sharing of animals and reduce redundancies between research groups, or the use of statistical tools to plan experiments and optimize breeding strategies for each experimental project.

Finally, I would like to advocate for the creation of a practical breeding guide at UNIL. Such a guide would offer concrete advice and incentives to researchers by explaining basic principles of animal colony management, while also providing useful tools and resources to implement an optimal breeding strategy. Information and transparency must be the cornerstone of this guide - with detailed annual statistics on the number of animals being raised, used and not used in experiments, along with the specific reasons for these decisions. When researchers are informed, they feel more concerned and get actively involved in improving their practices. Optimizing breeding management contributes to two of the three “R”s: reduction and refinement. My ultimate vision for the animal facility at Agora would therefore be to house fewer and fewer mice, while assuring that each animal contributes to the advancement of research.

**A wild type strain is a natural, genetically unaltered animal population. By contrast, genetically modified strains have undergone deliberate alterations in the laboratory to study the effects of these modifications on different biological aspects. Wild type mice are used not only as controls in studies on modified strains, but also to study different processes and characteristics, such as disease, behavior, physiology, and genetics of organisms in their natural state.*

More about 3R initiatives at the FBM

UNIL is strongly committed to putting the 3Rs into practice. In 2023, the university signed the Swiss “Culture of Care” charter – an initiative driven by the Swiss 3R Competence Centre (3RCC) and strongly supported by the Division of Research and Innovation of the Faculty of Biology and Medicine (FBM). This act demonstrates its firm commitment to adopting concrete measures to reduce, replace and refine animal experimentation at all levels.

Among these measures: the recruitment of a 3R coordinator at the FBM, Dr Stéphanie Claudinot, in 2018. Since 2022, the scientist has been working closely with both animal facility teams and researchers to develop and implement action plans to integrate the 3R principles into their daily activities. Her objectives also include breeding optimization and coordination at the FBM, for which she collaborates with several animal husbandry managers.

Find out more about Stéphanie Claudinot's projects in the next article, to be published in autumn 2024.