Home Office

NON-TECHNICAL SUMMARY

Breeding and Maintenance of Genetically Altered Strains with Validation and Refinement of Techniques Used in Archiving, Rederivation and Creating Genetically Altered Mice

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Cryopreservation, Reduction, Refinement, Genetic Alteration

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We support research into increasing the human health span by producing genetically modified animals as a resource for all our researchers. We apply the 3Rs principles of Reduction, Refinement and Replacement to ensure that the smallest number of animals suffer the least amount of harm to allow us to reach research goals.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Although medical advances have increase human lifespan, quality of life is often reduced by age associated diseases including diabetes, Parkinson's, Alzheimer's and many others. The research here looks at increasing how long people can stay healthy.

This project provides core services in support of the research on site by:

Establishment and maintenance of high health status colonies of Genetically Altered (GA) and wild type mice without genetic drift. Genetic drift is the change in frequency of particular genes in small populations over time due to individuals failing to reproduce. Central management of core colonies reduces excess breeding and allows production of large, age matched cohorts. This allows researchers to design experiments that can test several variables at once with one control group, instead of sequential experiments which each require a control group. This reduces the number of mice used in experiments.

Maintaining a bank of sterile male mice as a service for all users. Females used as recipients for GA or wildtype embryos, will not carry pups to term unless they have been mated. By pairing them with sterile males they become pseudo-pregnant, that means that embryos transferred into them will implant, but because their own eggs have not been fertilised they will not compete with the transferred embryos. Sterile males will be produced either by surgical vasectomy or by breeding a GA colony, the mice have no ill effects from the genetic modification, but approximately 50% of the males are sterile. We can tell which ones they are because the alteration is also linked to coat colour so there is no need to take tissue samples to genotype them. The fertility of the females is not affected.

Other Benefits:

- Freezing sperm and embryos of GA and wildtype strains provides a contingency in the event of damage or disease in the animal holding units.
- Creation of new lines of genetically altered mice in support of already approved research projects
- Quality control of reagents, equipment and protocols used in the other objectives. And quality control of frozen stocks of embryos and sperm.
- This project will investigate reports of new or improved methods, technology, equipment and strains that could increase the effectiveness or scientific potential of our current methods to achieve our other aims. We will test the validity, perform a cost benefit analysis and incorporate into our procedures those that will, on balance improve the welfare of the animals we use.

What outputs do you think you will see at the end of this project?

We will establish and maintain high health status wild type, immunodeficient mice and sterile male mice as a service for other approved projects. We will test equipment, procedures, alternative strains of mice and make quality-controlled reagents and media used in:

- production of sterile male mice
- embryo production and culture
- freezing and thawing of sperm and embryos
- revival of lines from frozen embryos and sperm
- IVF
- creation of new lines of genetically altered mice

We will also perform quality control tests on the frozen sperm and embryos

Who or what will benefit from these outputs, and how?

This project will reduce the number of mice needed for experiment by maintaining high health status production colonies free from undesirable genetic traits. This is important as the outcomes of experiments are more consistent if the subjects are all similar and this in turn increases the statistical significance of the results.

We will reduce the numbers of genetically modified animals kept alive and breeding through cryopreservation.

This project will also deliver a reduction in numbers of breeding animals needed by using in-vitro techniques for rapid colony expansion.

There is potential for reduction and refinement site-wide if new techniques for embryo production, cryopreservation, embryo transfer or creation of new genetically altered lines are demonstrated to be a

refinement or more efficient than our current methods.

Improvements in animal welfare are achieved by being able to import or export lines as frozen sperm or embryos thereby avoiding stress caused by live animal transport.

This is of potential benefit to other researchers and collaborators world-wide as we can send them frozen stock.

Potentially animal welfare could be improved by refining techniques.

Other projects or establishments that use genetically altered mice, but lack the facilities or expertise to manipulate embryos, freeze or thaw embryos and sperm or perform embryo transfers may benefit from the services of skilled technicians here.

How will you look to maximise the outputs of this work?

Our scientists often need large, age and/or sex matched cohorts to test several different variables. We will maximise the impact of our work by keeping the numbers of genetically modified animals bred to the smallest numbers consistent with producing the animals required.

Species and numbers of animals expected to be used

- Mice: 17,720

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use mouse eggs, sperm, embryos, juveniles and adults. Our scientists overwhelmingly use mice as models and our work provides these models for their use.

Typically, what will be done to an animal used in your project?

By far most of the animals used on this project will be used in breeding and maintenance of genetically modified mice. The majority of these will be sub-threshold.

Immunodeficient strains of mice are bred and maintained in a high health status unit. Protocols in place for husbandry ensure that the mice are unlikely to catch any diseases and therefore do not suffer adverse effects from their genetic alteration. Very few of the mice if any will require ear biopsies for genotyping.

Superovulation generally consists of two (occasionally up to four) injections of hormones and or other substances (e.g. antibodies) that have been reported to increase ovulation spaced approximately two

days apart. After this the females may be mated if fertilised embryos are required, but not if we need eggs for IVF. The female will then be killed and the eggs or embryos harvested.

Sterile male mice will be produced either by surgical vasectomy or by breeding a genetically altered colony known as Prm1 which is maintained in the facility. Half of the animals bred carry a dominant allele for overexpression of the Prm1 fusion protein, the males that carry the modification are sterile. The alteration is inherited from the females which are fertile. Following discussions with AWERB, it has been decided to phase out the Prm1 colony and replace with surgically vasectomised males as from a 3Rs perspective, it is felt that the reduction in numbers outweighs the refinement of no surgery.

All surgery will be carried out under general anaesthetic, mice undergoing surgery will get pain relief at the time of surgery and afterwards. Embryo recipients will have embryos implanted into their reproductive tract surgically or non-surgically. Surgical vasectomies will be performed using the most refined current method which is via the scrotum.

What are the expected impacts and/or adverse effects for the animals during your project?

Most of the animals will not experience more than mild severity. Although surgery is a moderate procedure, the animals are expected to recover promptly.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Two of the four protocols, 2 and 3 are classed as moderate, because they involve surgery, embryo transfers and vasectomies which may cause short lived post-operative pain or discomfort. This is expected to be less than 5% of the total number of mice used on this licence.

The remaining protocols are classed as mild, super-ovulation usually involves two injections of hormones which may cause mild transient discomfort. Superovulation will apply to approximately 8% of the mice used on this licence. The remaining 87% will be on a mild genetically modified breeding protocol and the majority are expected to experience no harm from their genetic alteration. A small number may require ear biopsies for genotyping.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This is a service licence concerned with the breeding and creation of GA lines for research, which can only be achieved by using live mice.

Which non-animal alternatives did you consider for use in this project?

Wherever possible non-regulated organisms, cell, tissue and embryo culture are used to learn as much as possible about molecular and cellular mechanisms before experiments using mice are performed.

Why were they not suitable?

The Institute's research relies in part on customised mouse models and the aims of this project are to create, breed and supply them.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimate for the number of animals used for breeding and maintenance of the immunodeficient mice has come from data extracted from the Mouse Colony Management system of the breeding performance of the colonies we maintain. I used data collected over several years on how long a stud male can be kept before his mating performance declines to estimate the number of vasectomies required or the number of sterile mice we would need to breed.

To work out how many super-ovulations we would need to do, we looked at how many quality control tests we have done over the previous licence and approximately how many we would need to do in the future based on how demand has been changing over time.

The number of embryo transfers is based on what proportion of the frozen stocks needed to be tested in-vivo in the past plus an expectation that work for external clients may increase in the future.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

When quality control testing frozen sperm by IVF we always have more samples on standby than we expect to use. If we have an unusually good superovulation, we can thaw the extra samples and test them as well as the planned ones. This means that we can get more tests completed with the same number of mice, so using fewer mice per test.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Super-ovulations are planned so that we use females of optimum age and weight to get the maximum number of high-quality oocytes from the smallest numbers of animals.

When selecting studs, males are picked between 2 and 6 months of age for optimum sperm production. To avoid wasting super-ovulated females by mating them to potentially sterile males, all studs are tested for fertility beforehand.

Numbers of embryos implanted per recipient female are carefully calculated to ensure the fewest number of transfers results in the highest number of healthy live offspring.

Following discussion with AWERB, it has been decided that although using the Prm1 colony is a refinement compared to vasectomies, the extra breeding of genetically altered animals and associated wild types outweighs the benefits of fewer moderate vasectomy surgeries. The Prm1 colony will be phased out which means that there will be a considerable reduction in the number of animals used on the breeding and maintenance protocol compared to the previous licence. We will therefore cryopreserve embryos from the colony, to supply other establishments if requested, or to revive the colony if the reduction/refinement cost benefit analysis changes in the future.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Surgery is performed by a small team of skilled technicians with excellent success rates. Analgesia is always given to animals undergoing surgery. Numbers of embryos implanted are optimised for the maximum numbers of healthy pups born from the fewest procedures.

Procedure success is monitored and reviewed, technicians receive regular refresher training to minimise the distress to the animals and ensure consistent results.

We have to maintain a group of sterile males because female mice will not maintain a pregnancy with implanted embryos unless they have been mated. The sterile males can be produced surgically or by breeding a strain of mice where 50% of the males are sterile. We can tell which males are sterile by coat colour, so we don't need to take tissue samples to genotype them. This colony does not show any harmful effects from the alteration.

When surgical vasectomies are performed we use the least invasive technique possible.

Some of our mice are immunodeficient, they are kept in a clean environment where the risk of them being exposed to disease causing organisms is low, so even though they do not have a fully functioning immune system this does not cause them suffering or distress. In a small number of strains, genetic modification may adversely affect animal welfare. Effects due to genetic alteration in strains authorised for this licence will be no more than mild. Close health monitoring provision of appropriate treatment under the guidance of the NVS and adapting husbandry routines to the needs of the animal will be used to ameliorate these effects.

Why can’t you use animals that are less sentient?

We breed immunodeficient mice as a service to other projects who could not do their work on immune systems without live animals.

We replace the use of animals with cultured embryos whenever possible, however we will need to breed mice with genetic modifications. Although it is possible to test embryos for the presence or absence of a particular DNA (deoxy-ribonucleic acid) sequence it is not possible to determine how this affects gene function without studying live animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Mice are picked up using non-tail handling methods which reduces stress.

All surgery will be performed aseptically. Mice undergoing surgery are kept warm during and after surgery with thermostatically controlled warming plates. Peri-operative analgesia will be given and maintained after surgery as long as is necessary to alleviate pain. Animals also receive an injection of warm saline solution to help post-surgical recovery. They also have opthalmic gel applied to protect their corneas, this can interfere with the grimace scale to measure distress. Instead our technicians are experienced in spotting changes in body posture or behaviour which would indicate distress.

Methods for producing genetically altered animals are constantly being developed. Any widely accepted techniques and innovation may be implemented when these would result in refinements of current practices. When advised of potential refinements we will seek advice from the NIO and if necessary seek additional training in order to incorporate them into our current protocols.

Immunocompromised strains will be kept in appropriate bio-secure housing such as isolators or IVCs

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

HO Minimum Standards for Aseptic Surgery. LASA Guiding Principles for Preparing and Undertaking Aseptic Surgery.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We get regular updates from NC3Rs on advances in reduction refinement and replacement. Our NIO produces a monthly newsletter and advises on how these can be applied in our work.