NON-TECHNICAL SUMMARY

Study of the mammalian hypothalamic pituitary gonadal axis.

Project duration
5 years 0 months

Project purpose
(a) Basic research

Key words
Neurons, Fertility, Testes, Hormones, Ovary, Hypothalamus

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

Objectives and benefits
Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?
This project is aimed at increasing our knowledge of the mammalian reproductive axis. Reproduction is of central importance in many aspects of human health and disease. Impaired fertility is an increasing problem in western society affecting around 10% of all couples. In addition, around 10-20% of
pregnancies are lost as a miscarriage during the first 12 weeks of pregnancy. Thus, an understanding of normal fertility and pregnancy is essential to develop potential clinical treatments for these problems.

Mice will be used to investigate the way in which fertility is controlled and identify genes that regulate the reproductive axis. To do this, we will generate mice that carry defined mutations and analyse the effect of these mutations on their fertility. We will also look at the cells in the brain that initiate puberty and how substances such as hormones regulate these and how they communicate with other cells in the brain.

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.

What are the potential benefits that will derive from this project?

This project is expected to provide novel information on the molecular processes that control mammalian fertility. It will advance our knowledge about the molecular pathways that control puberty and the production of eggs and sperm. This may allow the development of novel compounds that regulate the reproductive axis, which can be used as contraceptives or for the treatment of reproductive problems such as infertility or miscarriage.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Over the course of this licence, we will use a total of around 4,200 mice (approximately 860 mice each year). Of these, the majority (around 2,800) are used simply for breeding and these will have no detrimental effects caused by the genetic alteration. A further 400 mice will be used each year for the generation of the genetically modified mice and these will not have any surgical procedures performed on them. Surgical procedures (mainly removal of testes or ovaries) will be performed on around 650 mice with viral delivery into the brain be performed on around 350 mice.

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

In general, mutations that affect reproduction produce non-painful effects specifically on fertility and the mice are healthy and uncompromised in other functions.

The majority of mice will be used for collecting tissue samples for further analysis. These tissues will be taken from mice that have been humanely killed.
A small number of mice will have surgical operations under general anaesthetic to remove their ovaries or testes to ensure low levels of hormones from which we can evaluate any responses to treatments. We will also deliver substances directly into the brain via a small hole in the skull and allow the mice to recover afterwards. These studies will allow us to study the effects on fertility of substances normally found in the brain but which cannot easily get into the brain from the bloodstream.

Animals that have had surgery will be provided with appropriate pain relief and will be monitored regularly to check on their health. Any animal that loses too much weight or whose appearance indicates that their health is being compromised will be humanely killed. Adverse reactions to surgery will be minimized by using appropriate sterile techniques and are expected to occur less than 1% of the time. No surgical procedures will exceed loss of weight greater than 15%. All animals will be humanely killed at the end of the experiment.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Mammalian fertility is regulated by complex hormonal signalling that allows communication between several different body parts (e.g., brain, pituitary gland, and the testes and ovaries). It is not possible to copy this communication system using tissues growing in a culture dish, necessitating the use of live animals to study this system. In addition, sperm and eggs will only form properly in a whole animal. Moreover, the processes within the brain that regulate reproduction can only be studied in live animals.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We apply statistical methods to minimise the possibility (to less than 5%) of making either a false positive or a false negative conclusion, and as part of these calculations we can therefore ensure that we do not use more than the necessary number of animals to achieve statistical power and significance at the 95% level. For some studies, it is possible to monitor responses in the same mice over a period of time so that paired tests can be used which typically require fewer animals. Prior to undertaking a new study, we will perform Power Calculations to assess how many mice might be required generally using a significance level of 5%, a power of 80% and a least practicable difference between groups of 25% and if this indicates that the number is very large we will try to modify the procedure.

We will also keep the number of breeding pairs to a minimum (usually only 2) when mice are being maintained. We will only increase the number of breeding pairs to generate cohorts of mice for a specific study and reduce the breeding numbers as soon as possible after this point.

Before generating genetically modified mice, we will search public databases and publication records to ensure that they do not already exist elsewhere from where they can be obtained. Breeding will be optimized to produce only the type of mice that we need for experiments.
We will use sterile male mice for some of our work and we will purchased these from a commercial source and house them in an animal facility where they can be shared with other scientists to eliminate the need to duplicate having these mice in multiple facilities.

We maintain our genetically modified mouse lines as inbred stocks to minimize genetic differences that could contribute to variation in the parameters being measured. We also try to ensure that cohorts of mice are age and sex matched to reduce variability. Some mice are gonadectomised to remove variability caused by different levels of sex steroid hormones.

When GM mice are not required for future experiments, we will freeze eggs and sperm and embryos from the specific mouse line so that we do not need to keep the mice breeding unnecessarily.

**Refinement**

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Mice are an excellent model to study mammalian reproduction. The main physiological processes are conserved between mice and humans and the hormonal regulation of fertility in particular is almost identical between these species. Thus, knowledge gained from studies in mice can be directly applied to understanding human fertility. In addition, the ability to generate transgenic mice, in which a single gene has been altered or removed, provides a very powerful tool for studying the role of a single gene in reproduction. This technology does not exist for any other mammalian species. In addition, the short gestation period of the mouse allows us to study aspects of reproductive function relatively quickly.

We may also test whether administration of sex steroids on the skin can be used rather than by injecting under the skin as a less painful delivery route. We have also developed an ultra-sensitive method for measuring a hormone in the blood, which works with very small volumes of blood (5 ul) so that we can reduce the amount of blood taken from the tail vein.

We will retain existing ear clip tissue taken for identification purposes and use this for identifying the genetic make up of the mice [and thus eliminate the need to re-earclip for genotyping alone.

Transfer of embryos into recipient female mice to generate genetically modified mice will normally be performed using an NSET (non-surgical embryo transfer) device, which is a less invasive method than esurgical transfer of embryos.

Where possible, we will use genetically sterile mutant male mice (eg *Hiat1* mutant mice) instead of vasectomised males.