Home Office

NON-TECHNICAL SUMMARY

Regulation of embryonic lineages and stem cells

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Embryo, Pluripotency, Stem cells, Gastrulation

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?

The overall aim of this project is to understand how cells in the mammalian embryo are instructed and controlled during development, and how these cells can be harnessed and used to model development in tissue culture. This is important because our ability to generate specific tissues in culture would hold

great promise for replacement of damaged tissues, but we need to be sure that such tissues will function normally. Understanding the process of mammalian development is important for the design of strategies to generate specific tissues in culture and is also of wider scientific interest because the decision-making processes involved in forming an embryo are also commonly adopted for other biological functions.

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?

There are several reasons why we want to understand how mammalian embryos develop. Firstly, although in vitro fertilisation (IVF) programmes have helped many patients to conceive, disappointments still occur, usually during the early stages of pregnancy. Our research using preimplantation mouse embryos will enhance understanding of how the essential tissues are generated and regulated for normal development and implantation. This knowledge will also benefit production of embryos from livestock and as non-human mammalian models for research. There is already widespread use of stem cell lines and specific tissues produced from them in biomedical research. However, the full repertoire of cells in the body is not yet available in a dish. To generate the best culture models for disease modelling or drug screening we need to understand how they are formed in the developing embryo. Our research is directed at understanding the important early stages of the process. Finally, if tissues generated in culture are to be used to replace damaged tissue in patients, it is essential that the donor cells behave as intended and do not malfunction after transplantation, or turn into cancers.

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?

We expect to use a total of 13,250 mice during the 5 years of this licence. Of these, the majority (up to 11,500) may be genetically modified.

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?

Most of the mice to be used in this project are not expected to suffer any adverse effects. They will be maintained as genetically modified animals without any abnormalities for the production of early embryos to be used in short term analysis. Of the ~2,000 expected to be used in surgical procedures, up to 250 may receive transplants of cells or tissues that may form tumours. None of these tumours,

should they form, will be allowed to cause more than minimal discomfort. All animals will be humanely killed at the end of experiments and within one year if required only for the purpose of breeding.

Replacement

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

We replace the use of animals with cultured cell lines whenever possible. However, we sometimes need to breed mice carrying particular genetic modifications to provide early embryos for short-term experiments or to generate new stem cell lines. Unfortunately, there is currently no culture alternative that exactly mimics normal development. For this reason we sometimes transfer embryos that we have manipulated in culture into foster mothers for further development. We also occasionally need to determine the full repertoire of tissues that a cell line can produce in the context of a living body, in which case we may transplant the cells into an adult mouse.

Reduction

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

We minimise the numbers of mice we need to use primarily by making sure our breeding programmes are the most efficient for providing the genetically modified embryos we require. Wherever possible, and particularly for the purposes of training new staff and students, we use spare mice generated from our breeding programmes to mate for provision of early embryos to reduce the need to bring in extra mice. Our extensive experience in managing breeding programmes for the generation of embryos for research contributes considerably to the efficiency with which we can reduce the numbers of mice we maintain.

The methodologies for generating genetic modifications have now evolved sufficiently to enable us to delete specific genes in an inducible manner. This feature is particularly relevant for most of our work involving transgenic mice, since we are interested in the function of genes required to enable normal early mammalian development. Conventional deletion of the copies of such essential genes from both parents (known as 'homozygous null') in an embryo results in developmental failure. This means that homozygous null embryos can only be produced by mating male and female mice carrying one deleted copy of the gene each, and therefore, by Mendelian genetics, only 25% of embryos would be expected to be homozygous null. However, being able to breed mice carrying special modifications on both copies of the gene of interest that allow its deletion only when the embryo is exposed to a deleting agent means that all the derivative embryos from a mating have the potential to be made homozygous null. As a result, up to 75% fewer genetically modified mice need to be maintained to generate as many homozygous null embryos as would be required from animals carrying only a single deleted gene.

Amendment August 2020:

We have developed a modified method for studying the genetic requirements for tissue specification during embryonic development which involves observation and profiling gene expression of genetically modified embryonic stem cells that are integrated into normal mouse embryos to form chimaeras. This

means that we require fewer embryo recipient females for creating new genetically modified mouse lines than first estimated for this project. Instead of 1000 we will use fewer than 500 dams for this purpose.

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 the best animals for our research because they can provide all the genetic modifications we require. Furthermore, they are small and easy to breed, reaching sexual maturity within two months from birth and have the capacity to produce large numbers of embryos. Because of the biomedical relevance of our work, we need to use a mammalian system, and current scientific evidence indicates mice have a significantly lower potential for pain perception than other mammals. The majority of animals on this project are not expected to be subject to any pain, suffering, distress or lasting harm. A small proportion of our animals undergoing regulated procedures (<20%) will be subjected to surgery, which involves preparation for procedures required for making female mice receptive to transplantation of embryos, or for extending the period of development just before implantation (known as 'diapause'). All animals undergoing surgery will be provided with pain relief. Adult mice receiving transplanted cells to be tested for their potential to generate tissues will be carefully monitored daily for signs of ill health. If signs of ill health are apparent, or developing tumours are causing anything more than minor discomfort, the animals will be humanely killed.

Amendment August 2020:

We have developed a new way to increase our understanding of the roles of specific genes during embryonic development which involves introducing genetically modified and labelled embryonic stem cells into normal host embryos. Instead of creating a new genetically modified mouse line from these chimaeras, which involves allowing them to be born, and using them as a founders for breeding the new line, we take out chimaeric embryos during gestation and analyse the gene expression of all the cells. This allows the developmental potential of the genetically modified cells to be investigated in the context of a normal host embryonic environmental, as well as ensuring that the control and modified cells are exactly age-matched.