Home Office

NON-TECHNICAL SUMMARY

Early human development and the germline

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Primordial germ cells, Totipotency, Pluripotency, Epigenetic reprogramming, Stem cells

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

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 aim to understand early human development and to determine how mouse and human cells develop into sperm or eggs. The interaction between these cells and cells of the developing ovaries and testis is critical for developing viable sperm and eggs.

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?

Much work on cells forming sperm or eggs has been done on mice. Using the knowledge from past work on animals allows us to attempt similar human studies, which is essential because we know that some crucial differences exist between mouse and human development. Our focus will be on cells that appear in very early embryos that eventually develop into human eggs or sperm. For some studies, we use human stem cells, which can potentially be used to make sperm or eggs in a dish, including fetal ovaries and testis, which might allow us eventually to make human eggs and sperm in a culture dish.

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

Sperm and eggs transmit information to fertilised eggs that direct their development to adulthood. The precise nature of the information, genetic and non-genetic, remains to be fully elucidated. The nature of the information has long-term consequences in subsequent generations for human health and disease. Since the initial events occur in very early human embryos, we might also acquire knowledge of early human development. Advances in expertise from our research will also be informative for research on other areas of stem cell research.

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

The knowledge we acquire from our research will inform us of some causes of infertility, as well as about the cancers that develop from the cells that usually develop into sperm or eggs. We may also understand the causes of the current trend in the decline of human fertility and the potential development of approaches to arrest or reverse this trend. Environmental factors and pollutants also affect human fertility but how this occurs is unclear. The environmental factors can also induce non-genetic changes in cells that might be transmitted to subsequent generations with an impact on human health, including mental health.

The information transmitted through sperm and eggs has also been implicated with a role in the evolution of organisms. The research might elucidate the nature of the information that may have been important in, for example, the development of the human brain, which differs from that of chimpanzees, our closest non-human primate relatives.

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

As we complete each aspect of our research, we will aim to present it at scientific meetings and more widely through various outreach programs. We will also publish high-quality research in leading

journals to benefit advances in the field. We are also a part of the Human Developmental Biology Initiative (HDBI) established by the Wellcome Trust, a consortium of researchers working on all aspects of early human development, which provides opportunities to present our work to a broader group of researchers. Some events organised by the HDBI also offer opportunities to inform a wider audience, including the lay public, about advances from our work and their implications for human health and policy decisions on sensitive research areas on human embryos and the germline.

Species and numbers of animals expected to be used

- Mice: 2000

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 have generated transgenic mice with fluorescent reporters specific for the mouse germline. We will isolate germ cells and early gonadal tissues from these mice to investigate their development. In particular, we have developed methods to generate germ cells from pluripotent stem cells with these reporters and can conduct a significant amount of our research using these authenticated organs outsde a living animals (in vitro) culture models. We do, however have to obtain embryos and germ cells from mice for validation and to establish fresh pluripotent stem cell lines.

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

The animals are primarily used for breeding purposes to obtain authentic germ and gonads from transgenic and non-transgenic mice, and not for conducting research on animals directly in their bodies/organs (in vivo).

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

Animals produced under this protocol are not expected to exhibit any harmful phenotype.

We have not observed detrimental phenotypes in transgenic mice over several years. If any animal displays an adverse phenotype (mostly due to natural causes), the incidence will be recorded and the animal will be killed (Schedule 1).

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)?

We do not expect to see severe effects in our colony of transgenic mice.

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

- Killed
- Used in other projects
- Kept alive

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?

Human embryos are highly inaccessible for research. We use animals to test new experimental approaches, and the knowledge gained can be used to investigate similar events in precious human fetuses and embryos.

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

We have developed several approaches where we start with the mouse or human pluripotent stem cells. For example, we have already developed experimental procedures with stem cells that allow us to generate large numbers of cells that resemble precursors of sperm and eggs. We use these cells to carry out wide-ranging biochemical and molecular investigations leading to significant advances in knowledge. Consequently, we use far fewer animals for our research.

Why were they not suitable?

We have found many of our studies using stem cells are reliable for advances in knowledge.

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?

We have reduced significantly the number of mice we use from 4225 to 2000 for last 5 years and will continue to do so as we build new in vitro models.

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

As mentioned above, we can generate large numbers of early sperms/eggs from cultures cells. This has proved very important for research on mice and has enabled us to develop similarly in vitro models to study early human development and the germline. Thus, significantly reduced the usage of the animals for this project.

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

We use very few animals now because we have been able to develop in vitro methods using pluripotent stem cells. Importantly, the primary focus of our research is on early human development and the germline that has allowed us to use very few animals.

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.

We use GA mice (Oct4-GFP transgenic mice) in which we had introduced fluorescent tags associated with critical genes, which allow us to visualise and isolate pure precursors of sperm and eggs. Analysis of these cells will enable us to obtain information on authentic cells. We use the information to compare similar cells we make using stem cells in culture.

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

Developing precursors of human and mouse sperm and eggs have unique properties that do not occur in other vertebrates.

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

We have now reduced direct experimental work on animals. In the future, we would like permission to use Protocol 1 only for the Breading & Maintenance (B&M) procedure, which will be carefully monitored by the skillful technicians in the Institute animal house.

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

1. National Centre for the Replacement, Refinement and Reductions of Animal in Research (NC3Rs)

2. Breeding and colony management | NC3Rs

3. HOME OFFICE, Advice Note Animals Act 1986, Project Licence Standard Condition 18 notification

4. The ARRIVE guidelines: Home | ARRIVE Guidelines

5. The PREPARE guidelines: PREPARE (norecopa.no)

The NC3Rs is the best pratical guidance which has been followed through our project for last 5 years and will be a formal guidance for our next project B&M.

Another useful information is the 'User's Guide'. The booklet is informative for HO guidance; SC18 notification; study-plan; local rules for day to day practice etc, and also listed the useful links that we need to carry out the project as Breading & Maintenance (B&M).

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

While our use of animals has declined very significantly, we will continue to be informed about 3R, and use every available opportunity to reduce the use of animals.