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Home Office

NON-TECHNICAL SUMMARY

Oocyte chromatin determinants of offspring health

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Development, Epigenetics, Placenta

Animal types	Life stages
Mice	juvenile, adult, embryo, neonate, 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 wish to understand a newly-discovered route by which information from the egg is transmitted to the embryo, which depends on chemical tags (epigenetic marks) added to genes in the egg. We aim to understand whether these epigenetic marks are modified by maternal factors such as age and diet, and whether these effects persist to influence the development of the embryo or cause longer-term physiological outcomes.

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?

Our genes are wrapped by proteins to form chromatin to help condense the genetic information in the cell nucleus. How the chromatin is organised differs on genes that are active from those that are silent in any cell. In some cases, these different states can persist over the lifetime - a process referred to as 'epigenetic memory' - but it is also known that some chromatin states can be modified by factors such as nutrition or the environment.

The extent to which chromatin states in the egg or sperm are sensitive to extrinsic factors in a way that influences, or programmes, the development of the next generation is still poorly understood. Recent work has identified a new way in which epigenetic memory is passed between generations – a new form of ‘imprinting’ that depends on chromatin states in the egg – but very little is known so far about this new mechanism. We believe we have found a molecular explanation for this form of imprinting, finding that the controlling elements of these genes are of a class that could be sensitive to extrinsic factors. Our work will provide fundamental understanding of this newly described form of imprinting, answering questions such as the nature of the genetic elements involved, why it becomes restricted to the placenta, whether it is likely to be conserved, whether it is sensitive to extrinsic or physiological factors, what impact it could have on offspring development and health. This will provide important underpinning information from which to explore the existence and significance of this form of epigenetic memory in humans, and whether it is sensitive to maternal nutrition, age or procedures employed in assisted reproduction.

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

The key outputs will be a detailed understanding of a newly-described form of epigenetic inheritance in terms of how it is established in the female germline, maintained during preimplantation development and then restricted to the placenta; the functional impact of these genes on the placenta and through the placenta on the fetus; whether these genes have lifelong consequences in offspring; and whether these genes are particularly sensitive to deregulation from maternal factors, such as age, diet. All these outputs will help evaluate whether this form of imprinting could be conserved and what factors should be evaluated to test its existence and significance for healthy development in humans. These findings will translate into peer-reviewed publications, as well as public engagement and dialogue about the new science we discover.

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

(i) The academic scientific community, particularly in relation to developmental biology, epigenetics and reproductive biology. Our research will provide a detailed evaluation of the mechanism and developmental significance of a new mechanism of intergenerational epigenetic inheritance. Our research will also contribute to future studies: a number of new datasets will be identified and made available to other users to advance future research.

(ii) Staff and students supported by our lab, who will receive exemplary research training. Gaining technical skills and expertise to carry out research, in addition to other transferable skills, personal and professional development, will prepare them for careers in the academic or commercial research sectors or other related careers.

(iii) Funders, including the BBSRC and MRC. Our research underpins the delivery of strategic priorities for these funders.

(iv) Our research will equip policy makers with critical understanding of the impact and value of basic research, providing scientific rationale for the influence of diet and lifestyle on healthspan, reproductive biology and disease mechanisms.

(v) The general public including, but not exclusive to, students, teachers, patients, the local, national and international community. Benefits to these groups will include increased knowledge, understanding and awareness of our research on epigenetics, including the impact of environmental and nutritional exposures during development and its potential social and economic relevance.

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

We have many mechanisms in place to do this in addition to the immediate academic route of publishing research papers and presentations at international conferences. This includes a well-appointed and trained Knowledge Exchange and Commercialisation (KEC) team, and a similarly advanced and engaged Public Engagement (PE) team. We expect to develop collaborations in evaluating placenta physiology and programming. And with the principles of non-canonical imprinting established in mice, we would expect to develop collaborations with human geneticists interested in establishing whether an analogous epigenetic mechanism exists in humans.

Species and numbers of animals expected to be used

- Mice: 14,600

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.

The aims of the project are to understand epigenetic control of genes from the egg to the developing fetus, with a particular focus on how epigenetic memory from the egg controls the action of genes in the placenta. It is necessary to carry out this investigation in animal models because these genes affect organismal function in a complex way. For example, there is no cellular model yet for events start in the developing egg, are perpetuated in the early embryo before implantation, but have their effects mostly in how the placenta develops and controls growth and health of the developing embryo. We have been able to move a substantial proportion of our work into *in vitro* cell systems, thus reducing the number of animals used and refining the experimental approaches before applying them to mouse models.

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

The most common procedure in this project is the breeding and maintenance to produce adult or pregnant mice that will be killed via Schedule 1 to supply tissue for the aims described in this project.

We shall also be generating new genetically altered mouse strains using highly refined genetic modifications that will selectively affect genes in the placenta.

Smaller numbers of mice will be fed altered diets, such as high-fat diets, to evaluate the effect of diets on how the genes we are interested in are controlled.

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

The general type of genetically altered mice produced under breeding and maintenance in this project will be the type that use conditional gene ablation, which allows us to remove a gene specifically in target tissues (the placenta or egg) rather than in whole animals where constitutive ablation could have a severe phenotype, thereby avoiding adverse effects.

Mice fed altered diets, e.g., high-fat diet over a period of two to three months are expected to become mildly obese and diabetic, but these will be monitored to avoid development of harmful side-effects.

For mice that undergo surgery, mostly for transferring embryos, the duration of anaesthesia and surgery is short and the animals are expected to make a full and unremarkable recovery, although analgesia will be administered to mitigate short-lived pain.

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)?

Overall, the expected severity of this project licence is Mild, with fewer than 5% of animals expected to experience a maximum severity of Moderate.

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

- Killed

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?

The aims of the project are to understand epigenetic control of genes from the egg to the developing fetus, with a particular focus on how epigenetic memory from the egg controls the action of genes in the placenta. It is necessary to carry out this investigation in animal models because these genes affect organismal function in a complex way, which is not possible to recapitulate in purely cell-based systems. For example, there is no cellular model yet for events start in the developing egg, are perpetuated in the early embryo before implantation, but have their effects mostly in how the placenta develops and controls growth and health of the developing embryo. In addition, the impact of altered physiological states in the female, for example as caused by high-fat diets, on the development and quality of the egg and then into the offspring depend upon multiple cellular and tissue interactions that cannot be fully reproduced in cell-based systems. We have been able to move a substantial proportion of our work into *in vitro* cell systems, thus reducing the number of animals used and refining the experimental approaches before applying them to mouse models.

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

Inherent in our experimental strategy is the exploration of aspects of the regulation and cellular function of non-canonical imprinted genes in relevant cell culture systems, such as trophoblast stem cells (TSCs) or 2C-like cells that can be obtained from embryonic stem cells (ESCs), and this provides the information for the design of the *in vivo* genetic models. We are keeping fully aware of developments in cell-based systems, including organoids, and would adopt them where we can, if they prove reproducible and representative of the *in vivo* situation.

Why were they not suitable?

It is recognised that TSCs cells in culture do not faithfully maintain epigenetic states, they only partially mimic the full differential potential into extra-embryonic lineages, and cannot fully recapitulate the transition from before the fertilised egg to the development of a functioning placenta, and how these transitions could be influenced by physiological factors such as maternal diet.

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?

From experience of similar experimental designs in previous projects. With advice from the Institute statistician in relation to the minimum number of animals (data points) necessary to achieve statistically robust results in any procedure with a quantifiable outcome, including use of power calculations.

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

We have been able reduce the numbers of animals needed for these investigations because, for example, we have been able to develop highly sensitive methods for profiling the location of epigenetic tags in very small numbers of cells. We can also reduce animals numbers by making multiple measures from the same animal or sample, wherever possible. For example, current protocols for molecular profiling of tissues *ex vivo* enable us to obtain measures of gene expression, DNA methylation, and chromatin state in the same assay. As well as reducing the total number of samples, thus animals, needed to obtain these measures, obtaining multiple data from the same sample is a refinement in experimental design.

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

Use of power calculations of optimized animal group sizes based on comparable data from previous experiments and advice from the Institute statistician.

Minimising inter-group variability using controls of matching age, sex and genetic background.

Cryopreservation of strains when no longer required.

Use of colony management software that helps avoid overproduction.

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 mice in these studies because in this species we understand the most about where and how epigenetic tags are placed in the DNA to control the activity of genes, and because we are able to follow the fate of epigenetic mistakes during development in this species in a way that is not possible in other mammals, especially humans. We believe that the processes that put epigenetic tags in place

and how they control genes in offspring in the mouse are very similar to those in humans, so the mouse is a very informative model.

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

We are studying a form of gene regulation that is unique to mammals (imprinting) and its effects in tissues that are unique to mammals (the placenta). Much of the analysis will be done at an immature life stage, i.e., in tissues from mid-gestation conceptuses (*ex vivo* analysis of placenta), or will be done under terminal anaesthesia (e.g., placenta function assays).

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

Harm to animals is minimised by using sterile conditions, anaesthetics, humane methods of killing, and by targeting genetic mutations to the cells of interest (e.g., eggs, placenta) to avoid the possibility of whole-animal suffering.

Housing, husbandry and care conditions are provided by a dedicated Biological Support Unit (BSU), staffed by highly-trained animal technicians and overseen by experienced supervisors and NACWOs. The BSU enjoys permanent veterinary cover.

If, in rare circumstances, an animal has an unexpectedly severe response to a drug or operation, or where an infection develops, treatment is given where possible and, if necessary, the animal is humanely killed.

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

NC3Rs Arrive Guidelines.

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

We keep fully aware of developments in cell-based and organoid systems and would adopt them where we can, if they prove reproducible and representative of the in *vivo* situation.