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NON-TECHNICAL SUMMARY

Placental epigenetic programming

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Placenta, Epigenetics, Development, Gene regulation, Embryos

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

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?

The aim of this project is to gain an understanding of the importance of early events in genome regulation in the development of a healthy placenta during pregnancy.

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?

Pregnancy complications such as preeclampsia, miscarriage or preterm birth affect as many as one in four pregnancies, yet there remains a critical gap in our knowledge of the underlying causes. The placenta not only supports the growth of the baby, but helps the mother’s body adapt to pregnancy. A poorly functioning placenta can therefore not only impact the survival and health of the baby, but in many cases the mother as well. At present, we have a poor understanding of early events that lead to healthy placental development because these developmental milestones occur during the first weeks of human pregnancy.

Evidence supports that pregnancy complications are linked to changes in genome regulation in the placenta. In particular, epigenetics marks (chemical changes to DNA or its bound proteins) are key to setting up the active and silent regions of the genome during development. The patterns of epigenetic marks are unique to each cell type in the human body. Importantly, the establishment of these patterns are critical for a tissue to form correctly and function.

We want to understand the patterning of epigenetic marks in early placenta development. We hope to reveal how these marks direct the formation of a functional, healthy placenta, using the mouse model. Critically, mouse and human placentas contain similar cell types, epigenetic patterns, and developmental milestones, making the mouse a suitable model for this research.

We hope that our research over the next five years will lead to an understanding of how epigenetic patterning of placental cells in the early embryo support healthy placental development throughout pregnancy. This work will lay the foundation for our understanding of changes that may underlie complications of pregnancy.

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

The main outputs from this project will be a better understanding of the importance for epigenetic marks in directing placental development. We will share our findings through presentations at national and international conferences, publication in peer-reviewed scientific journals, and exhibitions at public outreach events, such as science festivals. Alongside publication, all datasets generated from our projects will be shared with the scientific community in public data repositories.

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

In the short term, these studies will provide important discoveries of the processes involved in early placental development. Our findings will help both clinical and basic science research in reproductive biology and epigenetics to generate new research questions. This work will form the basis for future research funding applications. These studies will also provide sequencing datasets from the early stages of mouse embryo development, when cell types are first being established. These available datasets can be further explored by other research groups to address fundamental research questions.

Importantly, these studies will help work towards an understanding how molecular changes early in development may lead to placental dysfunction and complications of pregnancy in humans. At present, there is a lack of clinical tools to provide early detection to help improve outcomes for complicated pregnancies. Hence, obtaining a better understanding of the underlying contributors to pregnancy complications will be a crucial step towards better supporting these pregnancies. In the long term, this work will help the public through improved care options for mothers and babies during pregnancy.

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

The outputs of this work will be maximised, in part, through collaborations. With a strong collaborative team, we will ensure the successful use of novel techniques and strong study designs. We will publish in high-impact, open-access journals, allowing us to widely distribute the knowledge generated by our work. We will publicise major findings in the press to further improve visibility of the research. When possible, we plan to publish negative results and detailed methodologies in open-access journals.

Species and numbers of animals expected to be used

- Mice: 4900

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 are using the C57BL/6 inbred strain of mice because this is the most common background for genetically modified lines of mice. C57BL/6 is the best known mouse genome for mapping of genomic sequencing data. The use of C57BL/6 will allow our data to be comparable to other studies in the field.

We have chosen to evaluate several stages of embryo development after the embryo implants into the mother’s uterus, as this is when placenta formation and function begins. We will assess epigenetic marks, gene expression and placental structure at various critical stages for placental biology. First, we will assess embryonic days 4.5 to 7.5 because at this time the embryo contains stem cells that will later derive all of the placental cell types and coincides with the patterning of epigenetic marks. We will then assess embryonic day 9.5, which is a critical time point in mouse placental development, when the cells that generate the umbilical cord link the baby to mother. Finally, we will evaluate embryonic day

12.5 because at this stage the placenta is fully formed, containing all of the necessary cell types for its function.

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

For animals used for this project, no procedures other than observational handling and matings are planned. Mated female animals will be humanely killed at predetermined life stages for embryo and placenta collections. Tissues will be used for generating genomic sequencing datasets to investigate genome regulation and for analysing cellular and structural changes in the placenta.

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

We do not expect adverse effects on the animals during this project because we plan to assess mice with a loss of gene function only at embryonic stages. There are no plans to evaluate these animals past birth.

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)?

Mice Mild 100%

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?

Studying the earliest stages of placental development in humans is logistically and ethically challenging because these events occur during the first few weeks of pregnancy. The experimental tools to study embryo implantation in the lab have not yet been developed. Hence, the mouse model provides a mammalian system in which we can study the molecular events during the formation of the placenta.

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

Human placental (trophoblast) cells can now be grown in lab, so we considered whether this would be a good alternative for this project.

Why were they not suitable?

Culture methods for human placental trophoblast cells are very new and still under development. As a result, these methods are not yet able to accurately model the early developmental windows of the placenta. Importantly, culture methods also cannot yet recreate the unique environment of the placenta. The placenta forms at the interface between mother and baby during pregnancy and contains cells from both mom and baby. Hence, the study of mouse development is essential in furthering our understanding of key steps in early placental development.

Importantly, prominent molecular features, such as the pattern of epigenetic marks on the placental genome are conserved between mouse and human. Mouse and human placentas have similar cell types, developmental milestones and cellular structure to separate the blood of mother and baby. As a result, our studies in mice will provide a critical foundation for future research into important regulatory events in human placental development.

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 prioritised investigating two epigenetic pathways in placental development. We will be studying knock-out (carrying two copies of a genetically modified gene) mouse models for the key enzymes in each of these pathways, which includes a total of 7 mouse lines. We estimate that each line will require 700 animals over 5 years to allow for enough matings to maintain the lines and perform experiments.

Maintenance of these mouse lines will be done by mating animals carrying a single copy of the genetically modified gene (heterozygous) to unmodified animals. This strategy is optimal for ensuring animal wellbeing and health. For each experimental mating, heterozygous animals will be mated together to obtain embryos. Embryo tissues will be used to generate molecular and sequencing datasets and to perform characterisation of the placental and embryonic features. Important considerations in this estimation of animal numbers are the frequency at which the correct genotypes can be obtained, the number of developmental time points being assessed in each line, and the number of experiments to be performed.

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

I have used the online experimental design tool provided by NC3Rs and the PREPARE guidelines to optimise of my study design, bias avoidance and sample sizes. I plan to collect embryos from

heterozygous female animals bred to heterozygous male animals. This heterozygous breeding strategy allows the collection of control and knockout embryos from a single pregnant female. Furthermore, using this approach, the genetic status of embryos is unknown to the experimenter during collection. The genetic status of each embryo is determined after samples are frozen or fixed, minimising any collection bias. Using Power Calculations, I have determined the minimum sample size needed for comparison of genomic sequencing datasets to be 3-5 per group. This will allow us to confidently identify changes in gene expression and/or epigenetic changes that are likely to impact cellular or tissue biology. Samples will be randomised for all molecular experiments to avoid technical bias. Evaluating cellular and structural changes in the embryo and placenta will be done using 5 samples per group to identify large, easily observable changes because we expect features to be dramatically altered in knockout embryos. The genetic status of each tissue will be unknown for experimenters assessing placental structure and cell composition.

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

My collaborators and I have developed cutting-edge adaptations of all sequencing methods. Rather than millions of cells required by the original protocols, we have methods that now only require hundreds to thousands of cells to obtain high-quality data. Without these advances, this project would not be feasible in mouse models.

In my postdoctoral work, I generated similar types of sequencing data from eggs and embryos collected from genetically modified mouse models. I also have performed pilot experiments in mouse embryos to demonstrate the quality and reproducibility of data that can be obtained from placental trophoblast cells. These studies have helped me accurately estimate the number of animals required to achieve our research goals.

The research project is designed with staggered aims, first focusing on one specific epigenetic pathway, followed by the other. This approach minimises the use of animals by avoiding unnecessarily prolonged breeding.

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.

Our plan is to use genetically modified mouse lines for breeding and maintenance. We don't plan any procedures or protocols that will cause pain, suffering or distress. Animals carrying the genetically modified targets selected for this study do not show any adverse phenotypes. Knock-out animals will be evaluated before birth.

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

To study placenta formation in pregnancy, it is necessary to study placental mammals. Hence, the mouse model is the least sentient model that we could use for these studies.

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

Stud males are those that are temporarily mated with females to obtain embryos at a specific stage. After pairing with a female, stud males cannot be returned to cages with other males, as this often result in fighting and aggression-related injuries. Hence, these males will be temporarily housed individually between matings. To ensure the wellbeing of these animals, we will singly house stud males for the minimum necessary time and utilise several refinement approaches, including the use of enriched environments and acclimatisation/rest periods between matings. To ensure the wellbeing of pregnant females after mating with stud males, females will be housed together until the relevant predetermined life stage.

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

For all animal work, I will follow the Laboratory Animal Science Association (LASA) guiding principles to ensure refined and considered protocols and approaches. I will adhere to guidance provided by NC3Rs strategies and PREPARE guidelines. I will frequently liaise with our Named Animal Care and Welfare Officer and Biological Support Unit facilities to review refinement of our approaches. We also plan to attend events and meetings, such as those provided by my organisation and NC3Rs, to stay updated on best practices.

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

We will stay up-to-date on advances in the 3Rs from the National Centre for the 3Rs (NC3Rs) guidelines and updates, as they evolve through the duration of the project. We will also use the Norecopa resources and University of Cambridge 3Rs search tool to find recent articles and relevant 3Rs news. We will stay attuned to the latest practical guidance from Laboratory Animal Science Association (LASA). In particular, we will closely follow any updates on the refinement and usage of genetically altered mice.