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NON-TECHNICAL SUMMARY

Cellular and molecular mechanisms of the mouse embryo development

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

mouse embryo, development, cell identity, stem cells, embryonic stem cells

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

This project seeks to understand early mammalian development with a special focus on how the different cell types of the embryo are formed and organised in the early embryo.

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?

Approximately 50% of human pregnancies fail at this early stage of development and we know very little due to the inaccessible nature of the human embryo at these time points. Therefore, gaining a greater understanding of how early mammalian development occurs will bring us insight into this significant medical problem. These studies will provide us with insights into how the major cell types of the embryo - the supporting placenta and yolk sac, and the embryo itself – are formed in the first week of development and importantly how and why mistakes in this process lead to loss of the embryo. We carry out work in the mouse system as the mechanisms and biology are likely to be directly relevant to human development and to understanding how pregnancies fail at these early stages. In addition, stem cells, which can be cultured in a dish, resemble the early cells of the embryo. As such, understanding the mechanisms that drive different cell identities in the embryo is also crucial for expanding our knowledge of how to direct stem cells to develop into the different cell types of the body. This will be critical to develop future approaches for regrowing, repairing, or replacing damaged or diseased cells, organs or tissues.

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

This project aims to answer outstanding questions in developmental and stem cell biology. The Principal Investigator and her research group are well known for running cutting-edge research, which is consistently published in high-impact journals and presented at conferences.

Expected outputs:

- publications in high-impact scientific journals

- conference presentations and papers

- new information in the field

- new experimental methods and approaches that could be used by other research groups in the future

- fostering of new international collaborations that will allow new approaches to current and future scientific questions

- training of scientists in responsible use of animals in research and mammalian embryology techniques

- data to support future funding applications and potential patent applications

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

Understanding development and cell identity in the embryo has many downstream benefits. In the short term it will improve knowledge of the mechanisms the early embryo uses to become specialised and grow. This knowledge will help us improve methods for working with, regrowing, repairing, and replacing damaged or diseased cells in the early embryo, which will likely impact in vitro fertilisation techniques. It will also impact on efforts to grow embryo-like structures using stem cells, which have huge potential to contribute to our understanding of development and to reduce the use of animals in research.

The immediate beneficiaries of these outputs will be the scientific community. Publications from this project will be published throughout the period of the project and scientists studying development, stem cell biology, reproduction, regenerative medicine and related fields will be the predominant beneficiaries of these publications.

In the medium to long-term, this project will expand our knowledge of how and why pregnancies fail. This will likely lead to improved methods for embryo culture, fertilisation, transfer and success in reproductive technologies, including fertilisation in the dish. We also expect that improvement of embryo-like structures development from stem cells will replace the use of animals in many areas, e.g. drugs and other chemical compounds testing or assessment of gene function during the development. Data obtained will also improve knowledge of the stem cell-based therapies.

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

- The Principal Investigator and the research group have several established national and international collaborations and are setting up new collaborations on an ongoing basis to maximise impact and dissemination.

- The outputs of this project will be presented at scientific conferences and published in high impact scientific journals to aid in knowledge dissemination. All publications will be open access.

- Dissemination of any approaches (successful or unsuccessful) and/or data (significant or insignificant) we may encounter in the duration of the project.

- The Principal Investigator will continue to recruit undergraduate and postgraduate students, as well as postdoctoral scientist to work on this project. These lab members will receive extensive training in mammalian embryology and the respectful use of animals in research. This knowledge and experience will be disseminated further as these scientists progress in their career and move between different laboratories and groups both academic and industrial.

Species and numbers of animals expected to be used

- Mice: 43 675

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.

Our group is investigating the mechanisms of mammalian development, from fertilization until the formation of the recognisable body after implantation in the womb. Mouse embryos are crucial in developmental biology research and are an excellent model to study events occurring during mammalian development, including many aspects of human development. Methods for mice breeding and maintenance, mouse embryo production, recovery and culture are well established and optimised, ensuring refinement and reduction in animal use. Our group has extensive experience with mouse embryology at pre- and early post-implantation stages.

Ultimately, we are interested in understanding early human development and disease. However, the use of human embryos is technically and ethically challenging. Therefore, the mouse embryo, which undergoes a similar developmental trajectory and allows for experimental manipulation is an excellent organism with which to carry out this research to investigate aspects of human development. The mouse represents an excellent balance between similarity to the human, size, experimental ability and cost. Our laboratory has a successful track-record of utilising mouse research to investigate and understand aspects of human embryo development.

It is important to add that, part of our scientific focus is the optimization of methods to create mouse embryo-like structures from mouse stem cells. Our success has led to a partial replacement of mouse embryos. However, these models do not yet fully capture the complexity of the mouse embryo, and therefore, it is, as yet, impossible to replace the use of mice in our current research.

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

Mice are used to generate embryonic tissue in our project. To obtain embryos, female mice will be administered two hormone injections (around 48 hours apart) to stimulate egg production and release. Hormones will be injected intraperitoneally (i.e. into the body cavity) or subcutaneously (i.e under the skin). This procedure, called superovulation, increases the yield of released oocytes, decreasing the number of mice needed to generate a set number of embryos for research. Subsequently, female mice will be placed with male mice for mating to fertilise the released oocytes. Following successful mating, embryos are obtained after humanely culling the dam and dissecting out the developing embryo.

We also plan to produce novel genetically altered mouse lines (without harmful effects), which help us understand the effect and role of specific genes. This procedure involves surgical or non-surgical transfer of genetically altered embryos into the womb of a female mouse, which in turn develop as normal pregnancies. Non-surgical embryo transfer will be used whenever possible as more refined method. However, in cases where it is not possible (e.g. transfer of embryos at very early stages, repetitive problems with non-surgical transfer efficiency that would not result in positive outcome), surgical transfer will be used. Surgical embryo transfer involves a minor surgical procedure performed under anaesthesia to transfer genetically altered embryos into the womb. During surgical embryo transfer mice are unconscious throughout the procedure and will experience short-lived post-operative pain and discomfort. The procedure will be performed in aseptic conditions to reduce risk of infection. To minimise any pain or distress, a range of available analgesia methods will be used. During non-surgical embryo transfer, embryos will be inserted directly into the uterus with a special device without any surgical intervention. Animals experience mild transient discomfort and no lasting harm. For embryo transfer (non-surgical and surgical), female mice are mated with sterile males to induce a

pseudo-pregnant state, which is essential for creating a womb that allows the transferred embryo to develop. It is planned to use naturally sterile males (genetically altered) by default as more refined solution. However, we predict that due to unexpected events (e.g. problems with availability), we may need to use sterile males generated by performing a vasectomy, where the tube carrying sperm is cut. We expect that this would be not more than 10% of sterile males used. These mice will experience minor post-operative pain and discomfort. This procedure will be also completed under general anaesthesia so that the animal will remain unconscious, and in aseptic conditions, to reduce risk of infection. To minimise any pain or distress a range of available analgesia methods will be used. Following birth, the mice with the genetic alteration are maintained and bred in order to keep the new genetic alteration and establish a mouse colony. Subsequently, the new mouse line would be used to obtain embryos for the experiments as described above.

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

For the hormone injections, the doses and routes used are well validated and are not anticipated to produce any adverse effects, beyond transient discomfort due to the injections, which will be intraperitoneal (i.e. into the body cavity) or subcutaneous (i.e under the skin).

Breeding and maintenance of genetically altered animals will have only minor or no effects that are more than minor or transient - animals are not expected to experience harmful phenotypes due to the genetic alteration, i.e. effects that would result in pain, distress, suffering or lasting harm equivalent to, or higher than that caused by the introduction of a needle in accordance with good veterinary practice (for example hair loss, swelling under skin, body deformations). For any new mouse line generated or received, welfare assessment with increased frequency of observation and monitoring will be performed to ensure that the alteration does not harmfully impact the animals.

Animals undergoing surgical embryo transfer will experience short-lived post-operative pain and discomfort. This procedure will be completed under general anaesthesia so that the animal will remain unconscious, and in aseptic conditions, to reduce risk of infection. To minimise any pain or distress a range of available analgesia methods will be used. Animals undergoing non-surgical embryo transfer will experience no more than mild transient discomfort and no lasting harm.

Male mice undergoing surgery for vasectomy (i.e. male sterilisation, a surgical procedure to cut or seal the tubes that carry sperm to permanently prevent pregnancy) will experience short-lived post-operative pain and discomfort. This procedure will be also completed under general anaesthesia so that the animal will remain unconscious, and in aseptic conditions, to reduce risk of infection. To minimise any pain or distress a range of available analgesia methods will be used.

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)?

Severity:	Hormone injections	Breeding and Maintenance of Genetically Altered Animals	Embryo Transfer	Vasectomy (male sterilization procedure)

Mild	**100%**	**100%**	**80%**	**0%**
Moderate	**0%**	**0%**	**20%***	**100%**
Severe	**0%**	**0%**	**0%**	**0%**

*Moderate severity will be experienced by animals undergoing surgical embryo transfer. Every effort will be made to maximize number of non-surgical embryo transfers, which will result in mild severity.

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

- Used in other projects
- 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?

This project aims to understand mammalian development, from fertilisation of the egg through to implantation in the womb and formation of the body up to day 10 post-fertilization. Many features of development are shared between mice and humans, including gene changes, embryo shape, cell identities and failures. Due to the significant ethical considerations of using human tissue for research, as well as the limited access to this resource, an alternative model is required. Mouse embryos represent the gold standard of mammalian development research as they resemble the human embryo in several key criteria. It has been shown that similar (homologous) genes, gene networks and mechanisms play role in early developmental of the mouse and human embryo.

Mouse embryos are also readily genetically manipulable, allowing for investigation of specific gene and cell functions in a way that cannot be done in the human embryo mainly due to ethical constraints. Special facilities, designed to keep and breed mice in the most ethical conditions are also present and will be used in this project.

Development at these stages involves the interaction of different cell types, including the embryo itself, the placenta, the yolk sac and the maternal womb. Simple model systems, including stem cells (i.e. cultures of cells with the ability to develop into many cell types of the body) and/or embryo-like organoids (three-dimensional tissues derived from stem cells) do not capture this complexity and, therefore, are unsuitable for the research outlined in this project. However, it should be noted that our group seeks, wherever possible, to answer questions in a system not requiring the use of animals.

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

We have considered the use of multiple non-animal alternatives:

1. Stem cell-derived embryo-like models (i.e. tissues based on structures derived from aggregates of different stem cell types) have been successfully developed by our group and others. These structures can partially replace or complement experiments utilising natural embryos when studying some developmental processes.

2. 2D stem cell cultures are widely used in our laboratory, not only for generating embryo-like structures but also as a preliminary model to study selected phenomena occurring during early mouse development.

3. Computer modelling to understand early development. Computational models can be used to make and validate experimental predictions without the use of animals.

Why were they not suitable?

1. Stem cell-derived embryo-like structures are being used to complement our research on mouse embryos. However, these embryo-like structures do not undergo normal development (i.e. do not develop from a single fertilised cell), do not contain all of the correct cell types, arrangement or genes of the natural embryo. Additionally, extraembryonic tissues that are also indispensable for proper embryo development (e.g. yolk sac) are not fully developed and functional according to our current knowledge.

2. Simple stem cell models, including 2D cultures, are useful to probe very specific questions (e.g. gene function in one cell type) but do not capture the complex, many cell type-nature of the natural embryo. Indeed, we are particularly interested in the interaction between the embryo and the surrounding tissues (placenta and yolk sac), which cannot be modelled in 2D stem cell cultures.

3. Computer modelling currently does not capture the complexity of the embryo, including how the cell types signal to each other and change their gene expression. This data can only be generated from further experiments in the embryo.

The above stem cell and computer models are routinely used and improved to complement our animal research. Those alternatives already partially replace or complement experiments utilizing natural embryos when studying some developmental aspects. However, as mentioned above, due to complexity of the natural embryo development and the changes in interactions between cells, which are not yet fully deciphered, it is still impossible to replace natural embryos in most cases.

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?

The project application and research plans have been broken down to 4 separate aims. We have divided each aim into the questions we would like to answer, and the anticipated number of animals needed to get this data. With our long-term experience in breeding and maintenance of mice and embryo generation, recovery and culture, we can estimate how many mice we would need. This number takes into our project aims, as well as, our specific protocols (for example breeding and maintenance of genetically altered animals).

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

During experimental design we use all available published data as well as our unpublished data to ensure that the questions are valid, novel and addressed in the best possible way, based on the current knowledge.

When designing experiments, we are considering different scenarios and outcomes and potential for the future directions. Number of embryos and mice needed to obtain a significant result is revised for each experiment. Subsequent planned experiments are always revised with the most up-to-date results. The experimental plans are modified as necessary. This is continually discussed between the licence holder and group members. We also run pilot studies to reduce the number of animals and embryos used when we start a new series of experiments. This is done to validate experimental design and/or new protocols that we plan to implement, and to ensure that the results obtained are measurable and specific, allowing us to draw conclusions in the future.

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

Methods for mice breeding and maintenance, mouse embryo production, recovery and culture are well known and optimised to ensure refinement and reduction in animal use. Our group and animal units have long term experience in successful mating, regularly obtaining high yields of embryos. This is assured by maintaining the wellbeing of the animals (for example, through proper handling, reduction of stress and diet) as well by implementing good practices that increase the efficiency of successful mating and superovulation. For example, there is maximum frequency of use for the males for the mating, mating is done in a 1:1 manner, males are continuously monitored, hormone injections are performed reproducibly (i.e. at the same time of day). This approach has reduced the number of failed matings (i.e. resulting in no embryos after superovulation, low embryo yields or bad quality embryos). Embryos are placed into experimental groups randomly. This ensures experimental and control groups are similar and increases experimental success.

Additionally, we use reliable reagents from globally recognized manufacturers and suppliers. This approach decreases the number of failed experiments, ensuring that the methods used are highly efficient and reliable.

As a good practice, close oversight of the group and experiments by the Project Licence Holder is always assured. This is carried out across the breadth of the project, including by regular meetings (at least once a week) to discuss the progress of the project and future experimental plans, ensuring that the experiments are planned well and they approach the problem in the most efficient way, and by regular meetings with mouse line managers (again once a week) to discuss status, health, availability

and performance of each mouse colony. Together this ensures that the mice are always bred and maintained according to best practices and with the reduction, refinement and replacement principals in mind.

Before proceeding to the actual experiment, we use available datasets whenever possible. For example, gene expression datasets, protein interaction databases and protein and gene features/functions databases. This preliminary step enables us to have additional insights regarding our experimental plans and questions and helps us to avoid redundant experiments.

We also run pilot studies to reduce number of animals and embryos used when we start a new series of experiments. This is done to validate experimental design and new approaches that we plan to implement, and to ensure that the results obtained are measurable and specific, allowing us to draw conclusions in the future. Finally, we also use computer modelling in several of our projects that use both mouse embryos and stem cells.

If there is a need coming from other groups, we are open to share animal tissues, including tissues from genetically modified mouse lines and post-mortem tissues, in order to further reduce overall mouse numbers.

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 experimental output are mouse embryos in different stages of the development. They are recovered from female mice mated with male mice. To obtain embryos for our research we use wild-type mice and genetically altered mice.

- embryo production protocol is a well-established and validated protocol. It brings only minimal suffering (i.e. mild severity) and is not anticipated to produce any adverse effects, beyond transient discomfort due to the injections, which will be intraperitoneal (i.e. into the body cavity) or subcutaneous (i.e under the skin). Transient discomfort results from restraining the mouse for the procedure for a short time (around 10-15 seconds) as well as from the injection needle.

- genetically altered mice bred and maintained for the project are not expected to experience harm due to the genetic alteration. All procedures related to the breeding and maintenance are expected to have a maximum of mild severity (and below that threshold in most cases).

- the only method currently available for allowing genetically altered embryos to develop is to implant them into the uterus of a pseudo-pregnant mouse. Thus, we cannot avoid this procedure. However, non-surgical embryo transfer methods will be used whenever possible as more refined method, where

the success rate matches that of surgical embryo transfer methods or is satisfactory. However, in cases where it is not possible (e.g. transfer of embryos at very early stages, repetitive problems with non-surgical transfer efficiency that would not result in positive outcome), surgical transfer will be used. Protocol for generation of genetically altered animals follows current standards and well-established procedures.

- we plan to use mainly genetically sterile males as an alternative to vasectomised males in generating pseudo-pregnant females.

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

Non-mammalian species (including Xenopus or Zebrafish) are often utilised in developmental studies and do share commonalities to both mouse and human development. However, these species diverge importantly in their developmental timing, gene expression and embryo shape in comparison to the human and therefore, are often inadequate models with which to draw conclusions on human development. The mouse embryo offers similarity to human development, while offering potential benefits.

To generate embryos, adult mice of reproductive age must be used. However, where possible, embryos are taken at the earliest point (from fertilization to day 10 post-fertilization when body plan is formed) to minimise life stage/sentience.

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

Our group and animal facilities staff have long-term experience in maintaining wellbeing of the animals and consistently implement new training and protocols. High standards in the facilities are maintained to ensure the animals are less stressed and well cared for (e.g. by provision of an enriched environment, acclimatisation when animals are moved into the facility, placement of animals into social groups). By implementing good practices, we also increase well-being of the animals and, therefore, mating efficiency (which leads to reduction in use). For example, frequency of use for the males is limited, mating is done in a 1:1 manner, males’ efficiency and behaviour are monitored, superovulation is performed in a repetitive scheme (hormones administered at a repetitive time of day). This approach reduces the number of failed matings, in turn reducing the number of animals required.

Establishment staff also have long-term experience in monitoring animal welfare and they ensure that every person involved in animal management in any way, must be first trained to the highest standards. If needed, increased monitoring and/or adequate pain management are implemented. Pain management will be achieved through the use of medicated palatable substances for voluntary treatment such as flavoured jelly, paste or milk shake liquid. We will get the animals used to the taste profile of the substances prior to the surgery so they will self-administer effective analgesia.

Humane endpoints are strictly adhered to minimise animal suffering during the project.

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

We follow the guiding principles on good practice for Animal Welfare. We adhere to the new ARRIVE guidelines (2.0), where possible. We also attain to the Lasa guidelines to ensure good practice on animal work, as well as the NC3Rs published strategy for improving animal welfare (see publication details below).

-Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/laban.1217

- LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. https://www.lasa.co.uk/current_publications/

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

We are regularly updated about advances in 3Rs by our Establishment staff (e.g. by Named Animal Care & Welfare Officers).

Additionally, we refer to available resources such as NC3R's (National Centre for the Replacement, Refinement & Reduction of Animals in Research) website page and 3Rs guidelines (https://nc3rs.org.uk/resource-hubs), LASA (Laboratory Animal Science Association) website page (https://www.lasa.co.uk/), RSPCA (Royal Society for the Prevention of Cruelty to Animals) website page (https://www.rspca.org.uk/), Norecopa (https://norecopa.no/databases-guidelines) and The Jackson Laboratory resources (https://resources.jax.org/).

We also take an active role in advancement of the 3Rs – for example we pioneered development of 3D stem cell models for mouse embryo development, which has been recognised by the NC3Rs (winner of the 2019 3Rs Prize).