Home Office

NON-TECHNICAL SUMMARY

Form and function during mammalian heart development

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Heart development, Embryogenesis, Cardiac Physiology, Regeneration

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

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 how the mammalian heart forms during embryonic development, focusing on mechanisms by which form and function emerge and interact. If we can understand how the heart forms in the first place, then we will be better equipped to repair or replace it when it fails during disease.

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?

It is important to undertake this work in order to address questions of fundamental biological importance but that also have clear clinical relevance. This relevance includes:

1. Congenital Heart Defects (CHD). CHDs are the most common type of birth defect, affecting 1:150 babies born in the UK (13 a day), however for over half of these cases there is an unknown cause. By studying how the heart forms we will gain new understanding into the mechanisms which regulate its development and thus identify new causes of CHD.

2. Regeneration. During a heart attack, billions of cells are lost from the heart leading to death or heart failure. Heart attacks cause 1.8 million deaths each year in Europe alone and whilst more people are surviving a heart attack those surviving subsequently go on to live with heart failure, another debilitating disease. In order to prevent this heart failure therapies are being developed which look to replace the lost cell by creating and implanting heart cells made from stem cells. In order to make the right cell type it is important we understand how to accurately make these cell types and therefore developmental biology acts as instruction manual in regards to how to make these cell types. Another regenerative approach is to reactivate mechanisms which cause the heart to grow in the embryo in the adult in order to stimulate new growth. If we can understand how the heart forms in the first place, we are better equipped to repair or replace it when it fails.

3. Cell based models of disease. To reduce animal experimentation and also gain biologically relevant human insight, it is important that we have reproducible cell based models. These models are based on using stem cells and can reflect at the cellular level what happens during embryo development. To generate accurate cell based models we need to understand how development happens in the embryo proper. Whilst our understanding of human heart development is increasing, human samples are extremely rare, thus it is fundamental we use model organisms to understand how the heart develops at an organ level. Once we have organ level reference data we can then refine and better trust the cell based models before using them to gain human relevant insight, such as understanding mechanisms of disease. For example, during heart disease there is an increase in the expression of genes which are required for heart development, therefore by studying developmental

processes and gene regulation in the embryo we can identify ways in which we can control these genes during disease.

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

At the end of this project we will have generated publications describing new insight into mechanisms controlling heart development and pathways which could play a role in diseases such as CHDs, heart failure, irregular heartbeat. This work will also highlight strategies in which we can create new cells to repair the loss of cells which occurs during a heart attack, thus aiding regenerative approaches.

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

Short-term outputs will benefit the scientific community by providing novel understanding in regards to form and function during heart development. Longer-term outputs will aid with the development of strategies to treat disease. Through the dissemination of our work at public events, such as Science festivals, we hope to increase understanding of our work with the general public.

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

To maximise the output of our work we will disseminate our work at conferences, science festivals and publications in open access journals. Datasets will be made publicly available at time of publication in accordance with the principles of our academic institution. Through collaboration we will also maximise the potential from our work by sharing new tools and samples. We will also publish/make publicly available any unsuccessful approaches to aid the community.

Species and numbers of animals expected to be used

- Mice: 5250

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.

Mice are the most appropriate mammalian species to be used in this project due to the similarities they share with humans in regards to heart development (i.e. cardiac morphology 4-chambered heart). Moreover, mice are a well characterised and widely used model of mammalian embryo development. Another advantage of using mice as a mammalian model is the availability of transgenic animals not available in other model mammalian species such as rats.

Adult mice will be required to produce, maintain and generate genetically altered embryos. Our research aims to explore how the embryonic heart develops, thus we will require pregnant females to collect or label embryos. Using both embryos and adult mice we will examine heart shape in different

strains of mice and explore how cells in early development contribute to the embryonic and adult heart. To generate new genetically altered mice we will require the collection and use of mouse eggs for fertilisation, microinjection and implantation. Neonate and Juvenile mice will be used to examine heart shape and how distinct embryonic cells contribute to the heart post-birth and prior to adulthood.

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

The main procedure performed in this project will be the breeding and maintenance of transgenic animals and the generation of embryos from timed matings. Embryos will typically be collected from pregnant females, during early development between embryonic day (E) 6.5 and 16.5. This will involve the pairing of female mice with a singly-housed stud males late in the afternoon. The following morning vaginal plugs will be check and any plugged females will be separated, and typically placed with another female to prevent singly housed animals.

We will perform intraperitoneal (into the abdominal cavity (IP)) injections to label/modulate cells during development. Typically this will require a single IP injection to a pregnant female during early development with embryos being collected before birth. As we will humanely kill the female to recover embryos this will typically only happen once to a pregnant female. Administration of labelling agents will also be administered either via oral (addition to drinking water/food), IP or gavage (feeding tube direct to the stomach) administration.

We will also perform temporary anaesthesia on pregnant females in order to conduct embryonic ultrasound recordings. Pregnant females will receive inhalant anaesthetic and be maintained on a heated stage with monitoring of heart rate. Whilst under anaesthesia ultrasound will be performed to characterise embryonic heart shape and heart rate which should require less than 45mins of anaesthesia. Having completed recordings the females will then either be humanly killed or allowed to recover. Following recovery embryos will either be collected at subsequent stages of development or allowed to litter down. This procedure would typically happen once to a pregnant female. A maximum of two recordings may be taken but the second recording would be non-recovery anaesthesia. During non-recovery ultrasound we may administer substances to modulate heart function whilst under anaesthesia e.g. isoproterenol. These substances would be given either into a vein or intraperitoneally.

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

The adverse effects experienced by the mice will typically be minimal. Intraperitoneal injections will cause mild transient pain during administration and oral garage will increase stress but should not be painful. In some cases the substances administered may lead to temporary weight loss, this will be for a limited duration as we will typically be working with embryos and the pregnant female will be humanely killed 4-8 days following administration.

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:

- Subthreshold = 74%
- Mild = 25%
- Moderate = 1%
- Severe = 0%

What will happen to animals used in this project?

- Killed
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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?

As our research focuses on the development and function of the mammalian heart during embryonic development. In order to understand how form and function arises during mammalian development, it is essential that live, intact embryos are used and therefore animal use is necessary. The proposed research aims to understand how the heart starts to beat and what influence this beating has on the development of heart shape and cell type composition. Due to the complex shape changes which the heart undergoes during development, the multiple different cell types which are required for its function and the signalling that occur in the intact embryo, such experiments can't currently be conducted in an cell-based or computer models.

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

We have considered and are using cell based models, human embryos and computer modelling as alternatives. These will reduce experiments conducted in mice. However these cannot yet replace the complexity of embryo development and human samples are extremely rare at these stages and have increased ethical implications.

Why were they not suitable?

To gain human relevant insight, human embryos would be the most suitable model to study. This, of course, is ethically challenging, and extremely limited due to early stage of development at which the human heart forms. We must therefore rely on standard animal model systems like the mouse. Cell based approaches will be used to look at how human stem cells can form different types of heart cells in 2D and 3D. This will allow use to explore specific cellular mechanisms and increase the relevance of our research as well reduce the number of animals used; however, these models do not accurately copy/model the multicellular 3D processes that occur in the intact embryo. Thus, we require the use of

mice to make direct observations and manipulations in intact embryos in order to provide a benchmark for the cell-based models we plan to develop.

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 estimated number of animals has been calculated based on the proposed number colonies we will require, our experimental plans (mainly relating to the generation of embryos from timed mating) and my 13-years of mouse colony management experience. Of the 5250 mice we estimate that 1000 mice will be used for experimental protocols (everything other than breeding and maintaince). However of the 4250 mice not used for experimental protocols around 40% of these will be used to generate embryos for experiments.

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

To reduce the number of animals used in this project we will take a number of steps.

Initially before moving to mice we will use previously published datasets to determine feasibility or cell-based models using human stem cells to refine our hypotheses, thus minimising the experimental questions requiring animal studies as well as maximising the focus and information obtained. We are also part of the NC3Rs cardiovascular network, which will provide opportunities to explore new biologically-relevant cell based models. We will use the PREPARE guidelines prior to initiating any experimental study to aid in the planning of each stage, and the ARRIVE guidelines to help in the design, analysis and reporting of all studies.

Longitudinal in utero ultrasound monitoring of embryonic heart development will provide data relating to both function (heart rate, blood flow) and form (heart size and morphology) in a non-invasive manner. This approach will allow multiple parameters to be measured from an entire litter (approximately 8 embryos) using a single animal without the need to kill the animals at each time point thus reducing the numbers of animals needed per experiment.

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

Efficient breeding strategies - to reduce the number of animals used for breeding we will manage our colonies actively with regular monitoring and varying colony sizes depending on current experimental plans. To optimise the number of animals used we will manage our colonies interchangeably e.g. rather

than ordering in fresh normal wild type mice (for breeding or experiments, we will use wild type mice generated from another one of our colonies. Where possible we will use breeding strategies which generate litters in which all animals have two copies of the required allele (homozygous), this will mean that all mice generated can be used for experiments or breeding. In other situations this breeding will not be possible due to early embryonic lethality when mice are homozygous, and thus these colonies will have to be maintained with a single allele (heterozygous) to enable breeding. We will use an internal tissue sharing distribution list to share mice due to unwanted genotypes or identify animals which could be used for experiments that would otherwise be culled. Randomisation will occur due to the varying genotypes of embryos within a single litter, meaning experiments will have inherent randomisation.

Sharing of tissue - given we focus on heart development, there are a number of other research groups in our institute who work on different organ systems during development such as the brain, blood and limbs. We will therefore share other parts of the embryos with these groups to optimise usage and reduce the number of mice generated.

Pilot studies - when establishing a new experimental plan we will use pilot studies to first determine whether we can detect any measurable differences. This will allow us to determine whether it is experimentally valid to continue with the proposed plan e.g. calculate sample sizes, determine severity level. Studies will be completed using randomisation and blinding.

We will also take advantage of our colony management system in order to identify where possible colonies that are already present within our facility and therefore reduce us importing/generating new animals. I am also part of the establishments colony management group that share best practice and guidance.

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.

Most of our methodology will be timed mating to generate embryos and thus animals will experience sub-threshold levels of pain and suffering. In some instances the animals will experience mild transient pain due to the administration of substances however this will be temporary. For genotyping we will use the most refined method possible, typically this will be ear biopsy which will cause the least amount of pain and also generate enough material for accurate result generation. Intraperitoneal injections of tamoxifen will be performed on adult and pregnant mice, which will cause mild transient pain but is required in order to provide rapid administration and generate results with biological relevance. If administration of tamoxifen is required in neonates we will look at using a more refined method such as orally from the end of a syringe. We will always liaise closely with our animal facility team and user management group to identify the current most refined approach.

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

Whilst other model species such as fish, toads, and flies do have hearts they have a different anatomy, which does not reflect the four chambered mammalian heart. They also undergo different process during embryonic development and have different mechanisms by which they regulate the heart beat. As we are exploring mechanisms which relate to human congenital heart defects it is fundamental that we work with a model system which is as similar as possible to the human. Mice are the most appropriate mammalian species to be used in this project due to the similarities they share with humans in regards to heart development (i.e. cardiac morphology 4-chambered heart). Moreover, mice are a well characterised and widely used model of mammalian embryo development. Another advantage of using mice as a mammalian model is the availability of transgenic animals not available in other model mammalian species such as rats. Mice are the least sentient models that we can utilise to address our experimental objectives in a biologically relevant manner and develop therapeutic relevance.

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

The majority of the procedures that we will perform have been extensively refined. For pregnant females we will aim to reduce all stress which will require a balance between monitoring the animals and leaving uninterrupted following timed mating. To reduced stress further we will refine our protocols for example with using tunnel handling. For the treatment of animals with tamoxifen as well as other substances we will monitor the best administration approach (e.g. IP injection, gavage), following internal tamoxifen administration guidance and liaising with Named Persons. Areas of refinement could include implementing a welfare scoring sheet following tamoxifen administration. We will also use a heated stage and warm gel when using ultrasound scanning. If animals do show signs of weight loss we will provide heat packs and soft food or a high-calorie diet to provide additional support.

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

We are regularly informed of the NC3Rs latest initiatives and publications by our establishment's HO licensing team. Our animal facility also has regular colony management and user meetings, where we are informed about current best practice. We will follow the PREPARE guidelines (Smith et al. 2018) as well as use the reporting metrics detailed in ARRIVE to ensure our experiments are conducted in the most refined way.

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

I am part of the NC3R cardiovascular network, and recently attended the launch event in London. We will attend Regional 3Rs symposia and other local webinars provided through our establishment. Our establishment also hosts colony management meetings and training events which provide information regarding the 3Rs and best practice. Another valuable resource will be our Named Persons, including the Establishment Licence Holder, Named Information Officer (NIO), Named Animal Care and Welfare Officer (NACWO) as well as Named Training and Competency Officers.