Home Office

NON-TECHNICAL SUMMARY

Long-term effects of a reduction in fetal oxygen supply on the maternal and fetal cardiovascular system

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

Developmental programming, Cardiovascular, Developmental hypoxia, Preeclampsia, Maternal and fetal health

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

The overall aim of this project is to assess the long term effect of a reduction in fetal oxygen supply on the maternal and fetal cardiovascular system. We are interested in this topic from a clinical perspective to develop maternal therapeutics that protect mothers and babies from developing cardiovascular disease in later life.

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?

Women often experience conditions during pregnancy that reduce oxygen supply to the developing embryo and fetus. The lack of oxygen (termed hypoxia) can permanently alter the cardiovascular system of the fetus, and can increase the chances of maternal preeclampsia. Both of these problems can increase the likelihood that the mother and child will develop cardiovascular disease later in life. Therefore, it is important to develop novel therapeutics that can protect the mother and unborn fetus from hypoxia and prevent heart disease in later life.

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

Data will be generated in the form of measurements of cardiovascular function which will be used in my laboratory, shared with academic collaborators and published in peer-reviewed journals. In the long-term, we envisage sharing the data with industry partners and the pharmaceutical industry to develop therapies for protecting humans from cardiac disease. All of the findings will be published in peer-reviewed leading scientific and clinical journals to ensure wide dissemination of the research findings.

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

This project has several expected benefits:

- Short term: Basic scientists and clinicians will benefit from understanding the long-term effects of low fetal oxygen supply on disease mechanisms, and maternal and fetal health

- Medium term: Clinicians will benefit from the identification of cellular targets for drug intervention in hypoxic pregnancies.
- Long term: People will benefit from the development of therapeutic interventions that protect mothers and babies from developing heart disease in later life

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

1. Dissemination of new knowledge at national and international conferences (posters, oral presentations, workshops)
2. Participation in public engagement activities, such as science fairs
3. Dissemination of multimedia material through digital media such as laboratory websites and social networks
4. Publication in high-impact open-source scientific journals
5. Publication of datasets in open-source repositories
6. Engagement with patient associations (societies, charities, hospitals)
7. Engagement with industry partners and policy makers to refine and regulate IVF culture media
8. Engagement with media to raise awareness of IVF research

Species and numbers of animals expected to be used

- Mice: 850
- Rats: 1000

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 mice and rats as surrogate animal models for understanding the effects of the developmental hypoxia on the human cardiovascular system. Rodents are an excellent surrogate model for studying humans because of their short lifespan, fast generation time and low husbandry costs. In some experiments, mice are a better choice than rats because their genome can be easily manipulated. On the other hand, rats are larger than mice, and provide larger tissue samples which reduce the amount of animals necessary for experimentation; this is particularly important for our in vitro studies. Lower vertebrate models are not appropriate for these experiments because their

cardiac physiology is very different to mammals, and many of the proteins we are interested are not well-conserved between mammals, fish, amphibians and reptiles.

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

Typically, pregnant rodents will be single-housed in standard cages and moved into an environmental chamber during pregnancy. Oxygen levels in the chamber will be decreased to simulate fetal hypoxia. In some cases, animals may be given therapeutics to protect the unborn pups and mothers from hypoxia, and this will be done via the mothers drinking water. We will then remove the animals and transfer them to normal cages 1 day before they are due to litter. Once pups have been born, we take regular measurements of body weight and closely monitor their health. Some animals will have minor procedures including ear biopsy, hair sampling and mouth swabbing. We may also take intravenous blood samples, or inject substances using standard routes (intravenous, subcutaneous, intraperitoneal). Blood pressure and cardiac function may be monitored in the mothers (during and after pregnancy) and the offspring. Some mothers and offspring may be surgically instrumented with telemetry systems to measure ECG's and and induce cardiac arrhythmias.

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

Pregnant rodents can experience moderate adverse effects from hypoxia, such as a transient or sustained reduction in activity or appetite, as well as pre-eclampsia like symptoms (e.g. increased blood pressure). Fetal hypoxia can cause offspring growth restriction, but this usually resolves after weaning. Other than that, there are no overt signs of any morphological or functional abnormalities in the offspring, and no signs of distress. Blood pressure measurements are non-invasive and have no adverse effects beyond handling, and echocardiography is done under anaesthesia, with only minor discomfort during anaesthetic administration. Animals may experience brief, slight discomfort with blood sampling and injection of substances, but no lasting harm. The telemetry surgery can induce moderate adverse effects due to the implantation of the probes into the body cavity (which can cause post-operative pain), but triggering of arrhythmias do not cause any noticeable distress or 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)?

Mice and rats: 68% mild, 32% moderate

What will happen to animals used in this project?

- Killed
- Used in other projects

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?

Animal models are necessary to investigate the long-term effects of fetal hypoxia because we need to make measurements across the life-course. It is not possible to do these experiments in humans because their lifespan is too long, and it is not ethical to alter the human fetal environment for experimental purposes. We can not use in vitro preparations because we are studying cardiovascular function at multiple life stages (fetal, neonatal, juvenile and adult) which can not be recapitulated in cell lines or organoids. The complexity of the vertebrate cardiovascular system and the longitudinal nature of our study also precludes the use of computer simulations, which are not currently capable of modelling cardiovascular development across the life course.

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

- Human volunteers or epidemiological data.
- In vitro preparations, including cell culture, organs on a chip and organoids
- Computer modelling and simulations
- Non-protected species such as fruit flies or nematodes

Why were they not suitable?

It is not possible to study humans for this set of experiments because we cannot control the developmental environment, and human tissue is; i) of limited availability, ii) rarely not already diseased and iii) nearly always subject to pharmacological interventions. We can not use epidemiological datasets because current repositories lack details on fetal and maternal conditions during pregnancy, and do not extend into late adulthood. We can not use in vitro preparations because we are studying organ development across the life-course, and it can not be suitably modelled using computer simulations. Lastly, we cannot use non-protected animal alternatives because we wish our findings to be clinically relevant to human diseases of the heart, and the use of other less sentient species, such as ectotherms and nonprotected species, is usually not appropriate for the main study animal as their hearts differ significantly from mammalian hearts.

Exceptions

There are some questions within our study that could be answered using cell lines. For example, if we identify drug targets, we could genetically manipulate cell lines (e.g. knockout experiments) to confirm that our targets are important before moving onto animal studies.

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?

Experimental design has been discussed with, and approved by, our statistical advisor. We calculated the minimum number of animals that we would need to produce statistically significant results. To do this, we analysed data from our laboratory and published articles to get an idea of how many animals are usually needed to produce a significant effect. Using this method, we estimate a sample size of 10 is sufficient for most of our measurements. This estimate will be updated and recalculated throughout the project as we generate new data. For longitudinal experiments, we've used our annual return of procedures data to estimate the number of animals that we will need to use for breeding.

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

We have taken measures to reduce the total number of rodents we use by performing intraperitoneal heparin injections prior to humane killing to prevent blood clots forming in the in vitro heart preparations. This procedure improves the success rate of our preparations by 50%, which reduces the total number of animals used.

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 - our laboratory has been breeding mice and rats for over 10 years. We have developed effective breeding protocols which usually result in an 80% success rate of pregnancy.

Pilot studies - we use pilot studies to confirm aspects of our study design, such as drug dosage

Sharing of tissue - we sample multiple organs from each animal after humane killing to answer parallel questions from the same animal

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.

In most cases, the level of hypoxia we will use is very mild (13% oxygen), similar to what you would find in high-altitude cities, such as La Paz (Bolivia). The more moderate levels of hypoxia (10% oxygen) that we use can cause more adverse effects, but these are usually transient for the mother, and do not produce any overt cardiovascular phenotype in the offspring. We have also chosen to use mostly non-invasive cardiovascular measurements to assess cardiovascular structure and function. Having thoroughly reviewed the literature, we are unaware of any other methods that could be used which would cause less suffering to the animal models we are using.

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

Many of our experiments are undertaken at immature life stages and ended before the animals are officially protected. However, because we are interested in the long-term effects of developmental hypoxia, we must also investigate later life-stages, including fetal, neonatal, juvenile and adults. We can not use less sentient animals because we want the results to be translationally relevant, and lower vertebrates have significantly different cardiac morphology and physiology. Where possible, we always use terminal anaesthesia for cardiovascular assessments. However, it is extremely important to obtain in vivo measurements without anaesthesia, because anaesthetic agents strongly affect the cardiovascular system and mask the responses we are trying to measure.

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

We use a specially designed hypoxic chamber that provides the animals with the most comfortable environment as possible. Rodent cages will include environmental enrichment (material to gnaw, refuges and nesting material), and solid floors with appropriate levels of substrate and appropriate lighting levels. It is not possible to house pregnant rodents in groups as we need to monitor food and water intake in order to characterise the phenotype of the rodent. However, once pups have been weaned, rodent will be housed in stable, compatible groups, taking into account sex, age, reproductive condition, familiarity, and prior group housing. We will regularly monitor the animals and use body condition scoring (BCS) to evaluate overall condition. The animals will be given analgesia when needed. Control rodents will be used to assess the normal body weight and BCS changes during pregnancy; this will be a separate group of pregnant rodents that will not be subjected to any procedure throughout the pregnancy.

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

We consult the PREPARE guidelines to design our studies (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence). This has allowed us to refine three areas of our study design; formulation of the study, dialogue between scientists and the animal facility, and quality control of the components in the study. We will continue to consult the guide as it becomes updated.

We also adhere to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines when publishing our research in order to maintain the highest standard of study design, statistical analysis and animal reporting.

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

We regularly consult information portals (e.g. www.nc3rs.org.uk, www.lasa.co.uk), published guidelines (e.g. https://journals.sagepub.com) and academic journals (e.g. Animals, Animal Welfare) to stay informed about advances in the 3R's, and to identify new techniques and protocols. We hold an account with the NC3R's where we receive regular newsletters, and our team continues to attend and present at NC3R symposiums.