Home Office

NON-TECHNICAL SUMMARY

# Environmental and placental contributions to pregnancy and life-long health

**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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

**Key words**

Fetus, Placenta, Fetus and maternal Health, Developmental Programming, Therapeutic interventions

| 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?**

To determine the impact of the environment of the mother and father on pregnancy outcomes (Aim 1), life-long health of the child (Aim 2), and the health of the mother after birth (Aim 3). To determine if interventions given to the mother, father or both improve pregnancy outcomes and life-long health (Aim 4).

**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?**

In developed countries like the UK, more than 17% of women develop complications during pregnancy. Such complications include low birth weight, development of diabetes in pregnancy (gestational diabetes), development of high blood pressure in pregnancy (pre-eclampsia) and premature birth. These complications can threaten the life and health of the mother and developing child. They also have life-long impacts on the health and wellbeing of the mother and her child. Women who develop diabetes or high blood pressure during pregnancy are more likely to develop diabetes and heart disease after birth. Babies with low birth weight are at greater risk of developing obesity, diabetes, heart disease and neurological impairments as they grow older. Pregnancy complications and their related life-long impacts are major emotional and financial drains on societies worldwide. Certain genetic factors and environmental conditions (like undernutrition, obesity, and stress) increase the risk of pregnancy complications. However despite this, we lack information on the pathways by which this may occur. The placenta is an essential organ that develops during pregnancy. It is responsible for transporting all the nutrients and oxygen a fetus needs to grow and develop. The placenta also produces chemical messengers that signal to the mother to support fetal growth and development. This programme of work will determine the importance of the mother's and father's environment for growth of the fetus and the placenta, as well as health of the mother during pregnancy (pregnancy outcomes; Aim 1). It will also follow the health of the child and mother after birth to see if there are long-lasting impacts (Aims 2 and 3). It will also identify interventions and treatments given to the mother, father or both parents to improve pregnancy outcomes and the life-long health and wellbeing of the mother and child (Aim 4). This work is important as there are no current treatments available to prevent the development of pregnancy complications or their related life-long health risks.

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

The outputs of this programme of work will be information on the impact of the environment of the mother, father, or both parents in the control of fetal development and pregnancy health (Aim 1). This

will be helpful in developing advice for couples who want to get pregnant or are having problems with maintaining a healthy pregnancy. By understanding the pathways altered by the environment of the parents, this work will also be helpful in identifying biological markers that can be used to screen for, diagnose and treat pregnancy complications. It will also provide information on the importance of environment/lifestyle of the mother, father, or both in determining the life-long health outcomes of the offspring (Aim 2) and the mother (Aim 3) after pregnancy. This will provide much needed information on the pathways that link the environment/lifestyle of the parents to the increased risk of the child to develop diseases like diabetes and heart disease in the years after birth. This will be useful in identifying people at risk of ill health from poor conditions during pregnancy and early life. It will also help us to know whether such conditions are already set during pregnancy, or arise after pregnancy (say when combined with poor lifestyle factors after birth). These findings will be valuable for the advice given to couples who are pregnant or planning pregnancy. Our experiments are designed in a way that we can also measure the extent to which a poor functioning placenta (an organ that develops to nourish the fetus) may play a part in the negative impacts of poor conditions in the parents on pregnancy and later health. These experiments will likely identify biological indicators that could be measured in the placenta at birth to help predict risk of diseases in later life. Identifying individuals at high risk would allow for closer monitoring and more targeted and earlier interventions to prevent diseases from developing. Finally by testing if interventions given to the mother, father or both, protect the fetus/offspring from developing ill health (Aim 4), we will find ways to prevent the harms caused. For example, our results will help people who work in health care to provide more specialised lifestyle advice and improve pregnancy health outcomes. We will also know if these beneficial outcomes are mediated by changes in the placenta, which will allow further development of treatments. For example, targeting therapies to the placenta to avoid any side effects on mother and fetus. All four aims will provide valuable information at many biological levels (cells, molecules, tissues and whole body) about which and how the environment/lifestyle of the mother and father instruct pregnancy outcomes and the life-long health. In the long-term, the outputs from this work are likely to reduce health care costs, the pain and suffering of affected individuals, and raise awareness of the role each parent plays in determining life-long health and disease risks, more generally.

For all the aims, the outputs of the programme of work will largely be in the form of scientific data which will be published as papers in high quality leading journals that are read by scientists and clinicians. The findings will also be presented at key conferences across different scientific fields which both scientists and clinicians attend. These activities will ensure the widest possible audience for the work. In addition, findings will be presented and discussed in lectures to undergraduate students. The findings will also be discussed with people in the media, politics, health care, companies, and at outreach events attended by members of the public.

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

The results generated in this project will have wide-reaching benefits for many scientific groups (including scientists and clinicians who study physiology, metabolism, reproduction and development) and will have great clinical, and public health relevance. Data generated will be used by researchers, including ourselves, clinicians (medical and veterinary surgeons), other health care professionals (for example, those working in assisted reproduction, midwives, nurses, health visitors) and potentially, in the long-term, by professional and government agencies (e.g. World Health Organisation, General Medical Council, Royal College of Veterinary Surgeons) and pharmaceutical organisations who may develop policies, diagnostic tests and therapies to combat sub/infertility, pregnancy complications and

later life health problems based on this study. In the long-term the outputs from this work are likely to reduce health care costs, the pain and suffering of affected individuals, and raise awareness of the role of the mother's and father's environment/lifestyle for life-long health and disease risks, more generally. The data generated will be used to:

- Design experiments by ourselves and colleagues in the scientific community in a wide range of fields, to further optimise human health, to reduce the burden of disease and to reduce, refine and replace the use of animals in experimental procedures.

- Identify environment/lifestyle factors in the parents that do and do not have potential health risks for the mother and offspring.

- Initiate human population and clinical studies in humans based on findings from our experimental studies in mice.

- Develop tools to diagnose, treat and prevent pregnancy complications and their related long-term effects.

- Revise life-style advice given to parents planning/currently pregnant.

- Provide advice to clinicians and other health care professionals on how to monitor, treat or intervene.

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

In the proposed work, we will ensure that we can obtain multiple datasets from each animal. In particular, we will make as many measurements as possible on an individual animal. At the end of the experiment, we will also obtain plenty of tissue and blood samples that can be used for additional projects. For e.g., tissue and blood samples may be used to develop a new method, answer a question outside of the direct scope of this project, or used to generate data for new work. We will maximise the outputs of this work by making data and methods freely available to others and we will also ensure rapid communication of findings through 1) open-access publication in the leading journals, 2) presenting our findings at key local, national and international conferences across different fields relevant to the research and at which scientists, clinicians and policy makers are in attendance, 3) taking opportunities to present and discuss findings in lectures to undergraduate students, with the media, politicians, clinicians, industry representatives and at public outreach events and 4) further outreach activities including publications in magazines, news articles, blogs, and via twitter / social media feeds.

**Species and numbers of animals expected to be used**

- Mice: Mice: 23,250

# 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.**

This project requires the use of animals in an integrative study of the body during development, pregnancy and later life. It requires exposing the animal to a genetic alteration and/or a change in its environment and measurements made on live animals and tissues and blood taken subsequently. It requires the study of the parents, fetus and offspring. Hence, this work cannot be fully replicated in vitro or in a cell culture system. This work would provide vital information on the importance of the environment of the mother, father and both for fetal development, pregnancy wellbeing, and offspring health. They are also important for knowing if interventions/treatments can improve pregnancy outcome and life-long health.

Mice have been chosen as the best non-human model for this study for several key reasons. This includes the knowledge that the patterns of egg/sperm and fetal development in mice are similar to humans. The genetic make-up of common laboratory mice allow us to undertake very precise investigations into the complex interactions between genes and the environment in determining pregnancy and offspring health. Such detailed study of these mechanisms in humans would be considered unethical. The structure of the placenta (the organ that develops to nourish the fetus) in mice is also similar to humans. Furthermore, the mouse also has a short gestation and short lifespan compared to other species which means that the proposed work can be completed within a shorter timeframe so that improvements in human health can be realised more rapidly. The mouse is also large enough to allow analyses of body functions (physiology), yet is small enough to permit complete assessment of tissue structure, which is not possible in larger animals. Finally, the expression of specific genes and the environment of the parents modified in a controlled way. This allows precise, highly refined information into the processes regulating fetal development, placental function, pregnancy health, and offspring wellbeing

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

Mice will be exposed to a genetic manipulation and/or a change in their environment. Manipulation of the environment for males will occur prior to mating and for females will occur prior to mating, during pregnancy and/or during lactation. Different combinations of matings will be set-up to assess the separate and combined impact of manipulations of the mother and father on pregnancy health, fetal growth and placenta function. Additional combinations of matings will be set-up to assess the health of the offspring and mother after pregnancy will be studied. Other work will involve testing the effectiveness of interventions/therapies in mice.

Animals in this project may be subjected to

•Genetic manipulations that alter key pathways within the whole animal or within certain cells/tissues (e.g. placenta), and tissue sampling (normally a ~2mm skin sample taken from the outer edge of the ear) to determine genetic status. This will involve breeding and maintenance of genetically altered mice and will tell us which genes/processes are important for healthy pregnancy outcome, as well as predisposition and protection of the mother and offspring from disease. The genetic manipulations will have been chosen to study development and physiology and animals will be exposed to mild severity

(causing no more than short-term, mild pain, suffering or distress to the animal, and with no significant impairment of their overall wellbeing or condition). We do not plan to generate new genetically altered lines.

•Environmental manipulations to the mother and/or father prior to mating, during pregnancy and/or during lactation. These environmental manipulations include dietary interventions (such as under-nutrition or diets containing high sugar and/or fat) and reducing oxygen levels (inhalation hypoxia). Animals may be exposed to the dietary manipulation for up to 12 months or low oxygen (inhalation hypoxia) for up to 22 days, but typically for a much shorter duration. This will inform which specific environmental conditions in each parent impact pregnancy outcomes and life-long health of the mother and offspring. The environmental manipulations have been chosen to study physiological responses, rather than extreme exposures/stressors and are not expected to exhibit any harmful phenotype. However, some animals will be exposed to moderate (instead of mild) severity, as they will be exposed to environmental manipulations and experimental procedures when they are pregnant.

•Environmental manipulations in the offspring and/or mother after birth. These environmental manipulations include dietary interventions (such as under-nutrition or diets containing high sugar and/or fat) and reducing oxygen levels via inhalation hypoxia. Animals may be exposed to the dietary manipulation for up to 12 months or hypoxia for up to 22 days, but typically for a much shorter duration. The environmental manipulations have been chosen to study physiological responses, rather than extreme exposures/stressors and are not expected to exhibit any harmful phenotype. These animals will largely be exposed to mild severity.

•Drugs including anaesthetics, hormones, stimulators and inhibitors of hormones and growth factor pathways, as well as imaging substances given to study how the body functions at specific times. Administration will either be through injection into vein, body cavity or under skin, in their diet/drinking water, or by the surgical implantation of a small infusion device (osmotic minipump) under the skin. Other than in terminally anaesthetized animals (where they will not be recovering from deep sleep prior to humane killing), the volume, route and number of administrations of the substance used will be the minimum needed to meet the scientific objective. These animals will be exposed to mild severity.

• Drugs including antioxidants (substances that can prevent or slow damage to cells caused by unstable molecules that the body produces), nutrients, and other possible agents with therapeutic potential given to study their effectiveness in improving pregnancy outcomes and preventing disease. Administration will be either through injection into vein, body cavity or under skin, in their diet/drinking water, or via surgical implantation of a small infusion device (osmotic minipump) under the skin. Other than in terminally anaesthetized animals, the volume, route and number of administrations of the agents used will be the minimum needed to meet the scientific objective. These animals will be exposed to mild severity.

•Embryo transfer experiments to begin to discriminate between the effects of changes in the eggs/sperm of the parents from those caused by a poor environment in the mother after mating. This will involve examining the development of the embryo in culture prior to implantation and then transferring embryos to recipient females with analysis of pregnancy and offspring health outcomes. We may also study the mothers after pregnancy, to see what impact there may be on her disease risk. As far as possible, the embryo transfer experiments to recipient females will be performed by non-surgical methods to minimise impacts on the animal.

•Alone housing for a maximum of 4 weeks to allow precise analyses of body functions (e.g. metabolism and food intake) or changes in food intake (including for pair-feeding when needed as an additional control group).

•Blood sample collection from an easy to access vessel.

•Fasting, so that precise measures of body functions can be determined (no more than 6 hours in pregnant animal and no more than 16 hours in adult mice).

•Litter standardisation or cross-fostering of offspring to different mothers. In the case of cross-fostering, this will occur in the first 48 hours after birth to maximise the success of fostering. We will monitor the mother until nursing behaviour has been displayed, or for a maximum of 1 hour. Cross fostering will take place in the home cage. The foster mother and pups will be handled with clean gloves to reduce the introduction of human odours and the pups will be mixed with the nesting material from the foster mother's cage.

•Non-invasive scanning/imaging or monitoring assessments to determine body composition, behaviour, metabolism and cardiovascular function on one or more occasions (e.g. time-domain nuclear magnetic resonance [TD-NMR], metabolic cages for up 72h, behavioural testing, ultrasound, tail cuffs; up to 10 over a 12 month period, but no more than one occurrence in pregnancy aside for TD-NMR).

•At the end of the experiment, each animal will be killed using the most humane method that is possible that does not prevent us obtaining good scientific data.

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

- Genetically altered mice used in this programme of work are not expected to exhibit any harmful phenotype (e.g. causing more than short-term mild pain, suffering or distress to the animal, and/or with significant impairment of their overall wellbeing or condition) but may show changes in their pattern of growth and development.

- The environmental manipulations have been chosen to study physiological responses, rather than extreme exposures/stressors and are not expected to exhibit any harmful phenotype. The environmental manipulations may cause reduced weight gain (e.g. with under-nutrition or with hypoxia which can be linked to reduced food intake), increased weight gain and/or mild features of metabolic disease (obesity, insulin resistance). These effects are no longer apparent when the manipulation is removed and/or often alleviate after a few days of the exposure (e.g. reductions in food intake normally only occurs during the first couple of days in hypoxia exposed animals). However, the effect of environmental challenges may be increased or seen for a longer period after cessation of challenge in mice with genetic alterations.

- There may cumulative impacts for animals undergoing different experimental procedures during pregnancy (e.g. reduced weight gain in pregnancy).

- If mice with gene alterations, environmental exposures and/or different experimental procedures during pregnancy exhibit any harmful phenotype, they will be humanely killed, or in case of individual animals of particular scientific interest, advice will be sought promptly from the local Home Office Inspector.

- Mice showing evidence of suffering that is greater than minor and transient or in any way compromises its normal behaviour will be humanely killed.

- Mice displaying weight loss that is not an expected outcome from the manipulation (altering growth trajectories that reaches 15% against age matched controls) will be humanely killed.

- Any mouse displaying rapid weight loss, measured on a daily basis, up to a maximum of 15% within a 7 day period, will be humanely killed.

- Any mouse displaying intermittent hunching or abnormal stance or reduced activity for a period of time not to exceed 24 hours, will be humanely killed.

- Any mouse displaying altered respiration (intermittent abnormal breathing patterns exceeding 1 hour) will be humanely killed.

- Any mouse displaying suppressed activity (subdued or isolated behaviour and reduced response to provocation for a period not to exceed 5 hours), will be humanely killed.

- Although highly unlikely, some animals may have an altered immune system making them more susceptible to infection. Animals with an altered immune status will be housed in a barrier environment thereby minimising the likelihood of compromising health.

- Mice are not expected to experience any lasting harm from being given hormones, stimulators/inhibitors of hormones/growth factor pathways, other drugs used in these studies or imaging substances. Novel drugs and other possible therapy agents will be tested to make sure they do not cause unwanted side effects that are harmful to the mice.

- Single-housing can be stressful for mice, but we will minimise the time each animal is housed alone. Where possible, animals will be re-housed in groups with their original cage-mates following a period of single-housing.

- In the unlikely event that pups are rejected in cross-fostering experiments the dam and her litter will be humanely killed.

- Animals are expected to make a rapid and unremarkable recovery from a surgical procedure involving anaesthesia within two hours. In the uncommon event that they fail to do so, or that the animal exhibits signs of pain, distress or significant ill health, they will be humanely killed unless, in the opinion of the Named Veterinary Surgeon, such complications can be remedied promptly and successfully using no more than minor interventions or a programme of enhanced monitoring and care is instituted until the animal recovers fully. Any animal not fully recovered from the surgical procedure within 2 hours (eating, drinking and returning to their normal behaviour) will be humanely killed. In the case of wound dehiscence, uninfected wounds may be re-closed on one occasion within 48 hours of the initial surgery.

- Animals anaesthetised for imaging (e.g., ultrasound) may feel disorientated as the anaesthetic wears off, but will experience no pain or lasting harm. TD-NMR scanning for body composition analysis and application of tail cuffs for blood pressure monitoring requires restraint, which can be stressful to mice, but animals will be trained / acclimatised to the equipment beforehand to reduce the stress.

• Previous work has largely assessed the effect of single parent manipulations on offspring growth, cardiovascular and metabolic physiology. We therefore do not know if there may be a stronger effect when environmental exposures are given to both parents (e.g. more severe body fat/weight gain and insulin resistance). Thus, careful monitoring of all offspring will be undertaken to ensure that they do exhibit any harmful phenotype. If they do exhibit an unexpected harmful phenotype, offspring they will be humanely killed, or in case of individual animals of particular scientific interest, advice will be sought promptly from the local Home Office Inspector.

• There can be impacts on the animals when they are undergoing different experimental steps when pregnant. However the number of steps will be kept to a minimum, and mice will be closely monitored to ensure there is no evidence of suffering that is greater than minor and transient. If so, they will be humanely killed.

**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 60%

Moderate 40%

Severe 0%

**What will happen to animals at the end of 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?**

This project requires the use of animals in an integrative study in vivo. It requires measurements to be made in vivo with subsequent in vitro analyses of tissues of mice and their offspring following environmental/genetic manipulation. It also requires measurements to be made in vivo with subsequent in vitro analyses in mice and offspring to test the efficacy of interventions/treatments in improving pregnancy outcome and life-long health. The outcomes of these studies are multi-factorial involving interactions in vivo between genes of the parents, the fetus, placenta and mother during pregnancy and between the mother and neonate/offspring after birth. Its study therefore requires the use of living animals rather than isolated cells, tissues and organs. However, ex vivo and in vitro

analyses (e.g. tissue culture, isolated organ function) subsequent to the in vivo measurements will allow us increase the data obtained and provide the comprehensive, integrated approach (from the gene to the systems level) that we are seeking in our programme of work. Consequently, answering the questions that we are addressing cannot be achieved in any other way than by using animals in vivo.

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

The sole use of cell culture and single organ systems would not permit investigation of the consequences of parental manipulation and the role of the placenta in maternal wellbeing, fetal growth and in the programming of disease in later life envisaged in the proposal. However, as a complementary approach, we will endeavour to use placental explants, trophoblast stem cells or cell lines, and the recently developed placental organoids as a way to reduce animal usage, in particular to help define precise molecular mechanisms and provide causal relationships (through, for example, genome editing in these culture systems). We can also use primary and established cell lines to test the contribution of specific molecular changes/pathways identified to be involved in the environmental programming of phenotype, but this can only happen after they have been identified from employing experimental manipulations in vivo. Current links with clinical researchers and elsewhere will help to translate findings generated under this licence, to humans.

**Why were they not suitable?**

Even with the best efforts in adapting experiments on cells, organoid and isolated organ systems in vitro, these set-ups offer limited benefit for understanding mammalian physiology and developmental processes in the later stages of pregnancy. In vitro set-ups do fully recapitulate the complex in vivo environment which changes over time with internal and external cues and involves dynamic communication between different organ systems, and signalling by circulating hormones, metabolites, and growth factors.

# 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?**

Numbers were informed on our previous work (published and unpublished/pilot experiments obtained over several years). This takes into account pregnancy success rates, numbers of small litters and natural neonatal survival rates.

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

We carefully consider each animal and what information it can provide, to maximise the possible tissue and data collection and reduce animal use. For example, for pregnancy studies, we retrieve the placenta, blood, and multiple organs from the mother and fetus, as much as possible, so that they can be used for multiple analyses across different projects. This reduces the numbers of animals used overall, increases the amount of data obtained from a single pregnant animal and allows us to examine links between the mother, placenta and fetus, thereby enhancing the quality of the information produced.

We also very carefully consider which control groups are necessary to address the scientific question and use power calculations as much as possible to ensure we will use an appropriate number of animals.

Assistance with experimental design was also obtained via web-based sources through the National Centre for the Replacement, Refinement and Reduction in Animals in Research (www.nc3rs.org.uk/experimental-design) and Prepare (https://norecopa.no/PREPARE).

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

Strains (types) of laboratory mice with known genetic status will be bought from known breeders or bred in-house, which reduces variation between animals. As far as possible, we ensure the breeding of colonies optimises the animals generated whilst minimising animal wastage. Only the number of breeders needed to generate the required number of offspring for a particular study will be set-up and scaled up or down, as required, as the litters are born and used. We will use control mice that are at the same age as the experimental mice and will use littermates (siblings) as much as possible. We will use mice that are genetically identical (i.e. sharing the same genetic background).

We will ensure the conditions of mouse husbandry are highly controlled (temperature, humidity, diet) and only parameters to be tested (e.g. diet) will be altered in a controlled way. Measurements will be taken at the same time of the day (e.g. to account for circadian effects on body functions). Where possible, initial checks of animal fertility and ability of the mother to nurse her pups will be made before the experimental pregnancy and particularly, when there is follow-up of offspring and maternal health.

We will randomly allocate animals to each treatment group and outcome measure. In designing experiments, controls and experimental groups will be run at the same time to avoid seasonal differences. We will perform pilot experiments, for e.g. to check the effectiveness of new therapies. Typically, controls and experimental groups are first compared with 4 animals in each group. If statistical significance is unlikely, no further animals will be used.

To maximise data gained from each animal, we will collect a large number of measures, as well as tissues and ample blood to provide a data and tissue/blood bank. This will enable us to carry out additional studies, including by collaborations - without having to use new cohorts of animals.

We also make extensive publication searches to ensure that we have a comprehensive, systematic knowledge of the literature in designing new studies and in identifying potential new collaborations that can use our archived material. We also go back to previously collected data from our animals, as it helps us to design new experiments, and may answer the novel questions with no extra mice needed.

# 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.**

Mice have been chosen as the optimal non-human model, for this study for several key reasons including i) their patterns of egg/sperm development, and embryonic and fetal development are similar to humans, ii) the genetic make-up of common laboratory mice permits unparalleled investigation into the complex environmental effects on offspring health and the pathways involved, iii) mouse models have become firmly established for analysis of environmental mechanisms involved in 'Developmental Origins of Health and Disease' applications and, iv) they have relatively short gestations and lifespans, which allows critical stages of development to be targeted and the effects of aging assessed, and v) the wide availability of modern gene deletion and knock-in technologies means mice are ideal for discovering the role of specific genes in mediating the effects of environmental influences.

The following methods will be used:

All surgery with recovery will be carried out under sterile conditions (free from harmful microorganisms). All animals will be monitored on a regular basis to ensure their welfare and protocols usually begin with the least invasive procedures and move onto more invasive experiments when positive results are obtained. Pro-forma recording sheets will be used during surgery to monitor variables (e.g. temperature, heart rate, anaesthetic dose) and post-operatively for the current severity score, food consumption and treatments with addition of free hand comments as required (e.g. recovery times, general demeanour) to monitor overall welfare over the course of an experiment. Routes, dosage volumes, frequencies, durations will be undertaken such that animals fully recover between administrations and will not suffer more than transient pain and distress and no lasting harm and there will be no cumulative effect of repeated administrations. In protocols involving food withdrawal in rodents, where possible food withdrawal will occur during the daylight hours, as this is better for the welfare of the animals. By archiving tissue for new studies and using equipment (e.g. metabolic cages [cages that all measurement of the animal's behaviour, food intake and body energy use], TD-NMR) with detection or recording devices that signal information and provide data without restraint, animal welfare can be maintained and data collection can be maximised while minimising the number of animals used experimentally. Of note, use of TD-NMR which is not invasive and does not require anaesthesia allows for longitudinal body composition to be measured in the same animal which reduces the numbers of animals needed. The use of metabolic cages which allows for highly resolved longitudinal and integrated assessment of metabolism, body weight and behaviour to be measured in the same animal which reduces the numbers of animals needed. The use of ultrasound which requires anaesthesia enables one to obtain longitudinal information on maternal-fetal blood characteristics and fetal development in the one animal which reduces the numbers of animals required.

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

Species that are less sentient or at an immature life stage would not allow us to address our key scientific question related to the role of the placenta in determining parental influences on pregnancy and life-long health. Less sentient species like flies or worms are not viviparous and therefore do not have a placenta or gestate. We use terminally anaesthetised mice for some procedures, and studies on terminally anaesthetised animals can help to identify physiological manipulations and/or intervention treatment in the preliminary phase. However, terminally anaesthetised animals do not provide the time scale or ability to track the developmental changes that underlie the longer-term programming of phenotype.

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

- Continually read papers in the field, including those which describe different methodological approaches to assess whether end-points measured should be adapted.

- Close supervision and accurate training records of personal licence holders to ensure their use of experimental animals always complies with the Home Office regulations, the project licence and to ensure there is minimal welfare costs for the animals.

- Close engagement with the Named Persons who might be able to help to ensure there is minimal welfare costs for the animals.

- Ensure and regularly evaluate records of animals used in experimental procedures, including post-operative care records and health monitoring of animals exposed to an environmental challenge to see whether any procedures need to be refined.

- Carefully record and examine mice in experimental protocols to ensure they do not reach the end of the severity band to which they are covered, and if so act immediately to ensure these are accurately reported and to avoid this in the future.

- Explore any unexpected deaths of any mice in experimental protocols to see what may have happened so that it may be avoided.

- Employ metabolic cages, TD-NMR and other scanners of genetically modified and environmentally challenged mice to refine animal use. Metabolic cages allow us to non-invasively and precisely evaluate many aspects of an animal's body functions, including food and water intake, energy use, behaviour, activity levels, and body composition and weight while they are normally moving around in the cage. Normally these assessments would be made on separate animals.

- As far as possible, we will use cage enrichment items (tubes) and group house animals, so that the quality of life of the animals is maximised. We will supply soft bedding and mushy food as necessary and in addition to weighing animals, we will monitor body condition scores (Ullman-Cullere and Foltz 1999 Lab Animal Sci) to provide further information on the wellbeing of the animals.

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

- Assistance when designing experiments by using web-based sources through the National Centre for the Replacement, Refinement and Reduction in Animals in Research (www.nc3rs.org.uk/experimental-design), ARRIVE (Animal Research: Reporting of In Vivo Experiment, guidelines for preparing publications; https://www.nc3rs.org.uk/arrive-guidelines) and PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence; https://norecopa.no/PREPARE and https://www.ncbi.nlm.nih.gov/pubmed/28771074)

- Laboratory Animal Science Association (LASA) guiding principles documents for aseptic technique for any surgical procedures (https://www.lasa.co.uk/current_publications/)

- Continually read papers in the field, including those which describe different methodological approaches and the latest advances to assess whether study design or end-points measured should be adapted.

- Close engagement with Named Persons and the Named Information Officer at my Establishment.

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

- Relaying information, ideas and strategies to refine our study approaches by discussing with members of the laboratory

- Regularly checking in with researchers in the laboratory to see if there are any queries relating to the licence and study designs.

- Regularly checking my Establishment website and search tool to check for any advances in the 3Rs and implementing these.

- Make a regular practice of reviewing our procedures to improve them from both welfare and scientific perspectives

- Continually read papers in the field, including those which describe different methodological approaches and the latest advances to assess whether study design should be adapted.

- Making use of the NC3Rs website pages https://nc3rs.org.uk/resource-hubs, 3Rs tools in-house, and externally resources, such as Norecopa https://norecopa.no/databases-guidelines