



Home Office

## NON-TECHNICAL SUMMARY

# Interventions in pregnancy: effects of obesity on maternal and offspring 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

### Key words

pregnancy, type 2 diabetes, cardiovascular disease, interventions, programming

### Animal types

### Life stages

Mice

embryo, neonate, juvenile, adult, 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 assess the possible benefits and risks of lifestyle changes and interventions (e.g., taking of medications or exercise) in obese females before and during pregnancy on both the immediate and long-term health of the mother and her offspring.

**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 western populations such as the UK, 50% of all women of child-bearing age are classified as overweight or obese. The National Institute for Health and Care Excellence (NICE) highlights the negative health risks of this on both the mother and her offspring in the womb and beyond.

One such complication in pregnancy is diabetes. If making changes to the woman's diet and lifestyle is unsuccessful in improving the condition, metformin (a drug in tablet form) is the next step. Whilst metformin is very successful in treating diabetes in the mother, little is known about its long-term effects on the offspring. Therefore, it is vital to investigate this important gap in our scientific understanding and assess if the benefits of metformin on the mother, outweigh any possible risks to her offspring.

We also aim to identify, assess and understand other types of interventions that could prove beneficial in improving the health of women and their children for example, exercise and the taking of antioxidants. Antioxidants are substances that slow down or prevent damage to cells in the body.

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

We intend to publish all findings (positive or negative) as research articles which will have been checked by independent scientists in the field. We will also present our findings at scientific meetings in the UK and overseas.

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

In the short term, it is expected that our published data will provide understanding of the ways in which interventions influence (positively or negatively) the health of the mother and her offspring. These data will inform health professionals (e.g., nurses, doctors, health visitors) about the effects of lifestyle choices and medicines in pregnancy for obese women. Data produced from these outputs will be used within future grants to fund further research into this area. These grants may include those for basic scientific research and/or clinical trials.

In the mid to longer term, these studies will result in collaborations, including clinical research groups and may benefit those such as the pharmaceutical industry. Our basic research will allow a directed approach to assess our findings in human trials which would further knowledge transfer and improve health.

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

We expect to publish in high quality journals and in order to do this our work will be assessed by independent scientists (those not working with our group) to make sure that the science is of excellent quality prior to publication.

Negative data (results that do not provide the expected outcome/result, or in the case of comparing two experimental groups, there are no differences for the measured outcome) usually forms part of all scientific publications. Therefore, negative data will not be dismissed but will be presented at scientific conferences and within scientific papers as is routine.

We will also engage with and disseminate information to other stakeholders including our funding bodies and the public. For example, in the past we have been involved directly with the funders (e.g., British Heart Foundation) by engaging volunteers and donors in events that allow them to observe / carry out the types of experiments we conduct as part of our research. These opportunities are likely to present themselves again in the future.

We will also continue to engage with schoolchildren and the general public through events such as science festivals. We are also very active on Twitter, with over 1200 followers which allows us to continue to disseminate the results of our work to a wider audience.

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

- Mice: 16,800

## **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 have been using mice to generate our model of obesity in pregnancy for approximately 12 years. During this time, we have consistently shown that this model has distinct similarities to many aspects of an obese human pregnancy. For example, the obese mice gain weight between their first and second pregnancies and they show signs of diabetes in pregnancy. We have also shown that similarly to humans, offspring born to obese mothers have a higher risk of disease (e.g., heart disease) in later life.

Mice allow us to study organs and tissues that would be either impossible or unethical in humans. For example, in order to understand what is going on in an organ at the level of the cell, we need to collect organs from the mother and/or offspring for us to perform our laboratory analyses. We also need to study effects of the mothers' diet on her offspring across their life course which are only feasible to animals with short lifespans such as mice.

### ***Life stages required***

#### *1. Female mice before, during and after pregnancy*

- As mice are only pregnant for 21 days this allows us to perform experiments in a feasible time-scale which would be impossible in humans where a pregnancy is much longer at 9 months.
- As our research aims to understand the impact of different environments during pregnancy, it is essential to study females before, during and after their pregnancy.

#### *2. Developing unborn offspring (embryo/fetus) at multiple stages of development (during pregnancy).*

- In order to understand how various maternal environments in pregnancy affect the growth and development of the unborn offspring (fetus) and the placenta (organ that allows the exchange of nutrients and oxygen from the mother to her unborn offspring), it is necessary for us to use tissues from the unborn offspring at different stages of development. Collection of such tissues would be impossible in humans.

#### *3. Offspring at various ages once they have been separated from their mother.*

- We need to use offspring at multiple ages exposed to one of four different pregnancy environments (healthy lean pregnancy, obese pregnancy and lean/obese pregnancy with intervention treatments such as drug treatment or exercise). We aim to study the impact of these different pregnancy environments on the future development of diseases such as type 2 diabetes and heart disease.
- Mouse models are critical for this research as they have a short lifespan (approximately 2 years), which allows us to study these diseases which develop with age. If we were to study the same effects in humans, we would have to follow the mother and child across an entire generation (30-50 years).
- This mouse model will provide understanding of the consequences of obesity in mouse pregnancy and the risk vs. benefit of such strategies to mother and offspring. This information will provide evidence to form a more targeted approach in studying the immediate and longer term impacts of obesity in human pregnancy.

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

Female mice (who will become mothers) will be weaned onto either a healthy diet or a diet high in fat and sugar and they will remain on these diets for their lifetime. This will generate our two main experimental groups, (1) control females (healthy pregnancy), (2) obese females. Both groups of mice

will then be mated for a first pregnancy to ensure that they are fertile and will rear their young successfully. After this first pregnancy, we check that the mice fed the diet high in fat and sugar have gained excess weight and fat mass compared to the lean healthy mice before mating both groups for a second pregnancy. The second pregnancy is known as our experimental pregnancy.

Females who become pregnant for their second time, with or without any interventions (e.g., drug treatments or exercise), will be allocated to one of three possible outcomes.

1. To study the effects of an obese or healthy pregnancy on the health of the mother and her unborn offspring.
2. To allow the female to give birth and rear her offspring. Her offspring will then be studied.
3. To study the direct effects for example of hormones, on the development of disease in the offspring. This outcome requires the surgical procedure, listed below.

Note, the interventions can be implemented either before pregnancy, during pregnancy, during lactation (period where the mother is suckling her young) or a combination of all three.

#### ***Measurements in females, before and during pregnancy.***

1. They will have their blood pressure measured using a non-invasive method that is similar to the way humans have their blood pressure taken, except it is done on their tail rather than on the arm. Typically this will be done on 6 occasions and maximally on no more than 8 occasions during the life course of the female.
2. To measure the degree of obesity, mice will be placed (still conscious) in a tube to have their fat and lean (muscle) mass measured inside an imaging machine such as an echo-MRI. This is like an MRI machine used by humans, but just on a much smaller scale. This is not invasive and usually takes less than 5 minutes. Typically this will be done on 7 occasions and maximally on no more than 10 occasions across the life course of the female.
3. To assess the heart health of the female and the health of her unborn offspring, we may choose to perform a non-invasive ultrasound scan. To prevent the animal from getting distressed whilst being handled and held for an awake procedure, we place them under a light anaesthesia (which is inhaled) and would typically last no longer than 2 hours. Mice will be gently awoken after the procedure and monitored closely afterwards. Typically this will be done on 1 occasion and maximally on no more than 3 occasions across the life course of the female.
4. Mice are usually housed together, but during and after pregnancy our mice are housed alone. We do this so that we can keep a closer eye on the mother (including her daily food intake) and her health during pregnancy. For offspring studies where females are required to litter, we will be able to check on her pups regularly and be certain on who is the mother of the individual pups in the cage.
5. In human pregnancies, mothers suspected of having diabetes will have a glucose tolerance test where they are given a sugary drink (glucose) and their blood glucose measured at multiple intervals over a 2-to-3-hour period. We will perform the same test in the mice but instead of a sugary drink, glucose will be injected into the peritoneal cavity (lower half of the abdomen) and glucose measured in

tail blood at timed intervals over a 2-to-3-hour period. Typically this will be done on 2 occasions and maximally on no more than 4 occasions during the life course of the female.

6. In order for us to assess the health of the female, we may take small quantities of blood from the tail which would allow us to measure important read outs of health such as blood sugar levels, insulin and cholesterol. Typically this will be done on 3 occasions and maximally on no more than 6 occasions during the life course of the female.

7. Some pregnant mice will receive an injection of dye (between day 12.5 and day 18.5 of pregnancy, that corresponds to mid to late pregnancy), which will allow us to measure how quickly embryonic cells divide. This injection is delivered quickly into the abdomen. The mother is then left to give birth and we are able to assess the results of the injection of dye in the offspring. This will typically be done only on one occasion and no more than two occasions.

8. In some pregnant mice a different dye will be injected (between day 12.5 and day 18.5 of pregnancy, that corresponds to mid to late pregnancy) that will allow us to determine if there is poor oxygen delivery to the unborn offspring and their placentas. This injection is delivered quickly into the abdomen and the mice are killed humanely up to 180 minutes later.

9. For those females that have not been used for point 8, at the end of the study we may perform ultrasound imaging to assess the health of the heart, placenta and fetuses in addition to their response following injection of substances that can speed up or slow down the heart. We will do this under inhaled anaesthesia and once the procedure is finished, the mother and her embryos will be humanely killed whilst still under anaesthetic.

10. At the chosen end time-point within pregnancy (for those mothers not entering at point 7, 8 and 9), the mother and her embryos will be humanely killed and all tissues will be collected following the confirmation of death and stored for future laboratory analyses. These tissues will be available to our group and collaborators upon request.

11. Females that are required to litter to allow for the generation of offspring will suckle their pups until weaning (when her pups are 21 days old). After this she may undergo a glucose tolerance test (as described in point 5) prior to her being humanely killed and her tissues collected and stored for laboratory analyses. Her offspring will be housed together grouped by sex, and fed either a standard (healthy) or obese diet and will undergo procedures listed below, under the heading 'Offspring'.

### ***Surgery in pregnancy***

A small number of females will undergo a surgical procedure during pregnancy. This procedure would be performed under general anaesthetic with all the surgical precautions taken pre-, during- and post-surgery. The surgery time is expected to be short (approximately 30 minutes in duration, from sedation to awakening). During surgery, a small incision in the abdomen would be made to expose the uterus containing the fetuses. At this point a very small volume of liquid containing a substance (for example a hormone) will be injected either into the amniotic sac where the fetus is contained or, into the fetal brain. Following injection, the incision would be closed with stitches or staples and the mouse allowed to regain consciousness by removing the anaesthesia. She will receive appropriate pain medication and will be observed by the scientist performing the procedure and by animal technicians. She will then

be observed daily to ensure she was in no pain and that the wound was healing as it should. She will be monitored daily to ensure she has a normal birth.

Once recovered these females will be left to litter and bring up their offspring up until weaning (when the pups are 21 days old). At this point the mother may undergo a glucose tolerance test (as described in point 5) prior to her being humanely killed and her tissues collected and stored for laboratory analyses. Her offspring will be housed together grouped by sex and fed either a standard healthy diet or obese diet and will undergo such procedures listed below, under the heading 'Offspring'.

### **Offspring**

A lot of the non-surgical methods used in the 'Measurements in females, before and during pregnancy' section will also be carried out in the offspring as we try to gather an understanding around their heart health and their risk of developing obesity and diabetes.

- (a) Assessing fat and lean mass by the method detailed in point 2 above. Typically this will be done on 7 occasions and maximally on no more than 10 occasions across the life course of the animal.
- (b) Measuring blood pressure non-invasively on multiple occasions throughout their lifetime by the method detailed in point 1 above. Typically this will be done on 6 occasions and maximally on no more than 8 occasions during the life course of the animal.
- (c) Glucose or insulin tolerance tests which require injection of either glucose or insulin by the method detailed in point 5 above. Typically this will be done on 2 occasions and maximally on no more than 4 occasions during the life course of the animal.
- (d) Blood sampling by the method detailed in point 6 above. Typically this will be done on 3 occasions and maximally on no more than 6 occasions during the life course of the animal.
- (e) We have the capability of measuring offspring food intake by weighing food daily within the animal's home cage. We may do this by housing the offspring on their own (single housing) so there is no competition between multiple mice for food. This will provide us with an accurate result on an individual mouse's drive to eat. Typically this will be done on 3 occasions and maximally on no more than 6 occasions during the life course of the animal.
- (f) Offspring heart health (using echocardiography) will be assessed using a non-invasive ultrasound scan which will be performed under inhalation anaesthesia (and the mouse will remain anaesthetised throughout the procedure). Typically, the procedure takes no longer than one hour. Animals will be awoken at the end of this procedure. Typically this will be done on 3 occasions and maximally on no more than 6 occasions during the life course of the animal.
- (g) Similar to the dams' section, we will also assess offspring heart health in response to a range of drugs that for example, can speed up or slow down the heart rate. Animals will be sedated for the procedure and be killed humanely whilst still under anaesthesia and tissues collected for storage within our lab tissue bank.
- (h) To further understand the risk of the offspring developing diabetes, we will perform a procedure called a euglycaemic clamp. In this procedure the offspring will be fasted overnight, and their blood sugar (glucose) levels recorded. Blood glucose levels are measured from a small drop of blood from

the mouse's tail. The mouse will then be anaesthetised (and will remain anaesthetised throughout the procedure) and a known quantity of insulin (hormone) will be injected into the abdomen. This will lower the blood glucose levels. Using a cannula inserted into the tail vein, glucose will then be slowly administered to increase and then maintain blood glucose levels at its starting value. After this procedure the mouse will either (a) killed by an overdose of anaesthetic (b) injected with a substance to kill the animal while preserving organ and tissue structure while still under surgical anaesthesia.

(i) Offspring that have not come from point 6 and 7 will be humanely killed at the end of the experiment and their tissues collected and stored for future laboratory analyses for us by our group or collaborators upon request.

**A small proportion of surplus offspring (that would otherwise not be used/culled for statistical reasons) and that have only had minimal phenotyping e.g. weighing, body composition or non-invasive blood pressure measurements may be transferred to other collaborative projects for further specialist techniques for which we do not have expertise, i.e. feeding behaviour, autonomous feed preference. This is important to ensure we maintain good 3Rs practice and would avoid the need for more pregnant mice and offspring to be generated.**

**Any surplus genetically altered mice that have not undergone any regulated procedure will also be made available to other users to maintain good 3Rs practice.**

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

As the large majority of work is non-invasive there are very few procedures within this license that would be expected to cause any impact or adverse effect.

However one procedure that would be expected to cause adverse effects would be surgery. The surgical procedure will be carried out under anaesthesia and pain will be alleviated through the use of pain relieving medication. We have kept the surgical procedure short to reduce any complications during anaesthesia. Complications surrounding wound healing (where the animal was cut open) are expected to be rare, but where these cases occur we will ensure their pain is adequately controlled using pain relief. In the rare cases where the wound re-opens we will place the animal back under a short anaesthesia to repair the wound, we will only repair the wound once. If neither of these improve the outcome, the animal would be humanely killed. Post-surgery monitoring will be carried out by the experimenter and animal technicians multiple times per day in the first few days post-surgery and all mice will be provided with soft bedding to provide optimal conditions for quick / uncomplicated healing and housed together where possible.

**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 = 98%



Moderate = 2%

### **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?**

Modelling obesity in humans is highly complex as there are a number of physiological alterations that are impossible to model in cell culture systems. A cell culture system involves growing cells in a dish within the laboratory under tightly controlled conditions. The growth of these cells can be monitored and expanded to accommodate the experiment required at the time.

As we are studying long-term health impacts of obesity in pregnancy on the offspring, the short pregnancy (21 days) and lifespan (approximately 2 years) of the mouse model means that we can study disease progression within a short time frame (i.e., for duration of a project). Typically, in humans this could take half a century to study.

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

Based on findings generated from our previous project license we were able to replace some of our animal work and use cell lines generated from humans and animals to further investigate findings from our animal models in cell culture (where cells are grown in a small dish in the laboratory to increase the number of cells available to do experiments). Cell lines from animal models are still classed as a replacement as they have been immortalised, therefore no further use of animals is required. We will continue to do this as part of this PPL.

### **Why were they not suitable?**

These non-animal alternatives are not suitable for total replacement of animals within our project as we still cannot model pregnancy or the complexity of obesity in a cell culture system.

## **Reduction**

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific**

**objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

**How have you estimated the numbers of animals you will use?**

We have based the estimated number of mice on the numbers and findings from our previous and current project licenses and our Home Office returns which we return annually.

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

Multiple users within our research group will coordinate their projects to work on different organ systems while using the same mice. This will not only allow us to gain whole body information on a single mouse, but significantly reduce the number of animals used. This method will also improve data quality as the results from different organ systems can be compared within the same mouse.

To further reduce the numbers of animals used and to replicate what happens clinically whereby only obese women would be offered interventions in pregnancy, we usually only implement intervention strategies in obese females.

We would also utilise where possible banked tissues and cell lines from wild-type and genetically modified animals in the first instance prior to generating any new cohorts.

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

As we only require the female offspring (from our in-house stock breeders) for our pregnancy experiments, the male offspring will be made available to other users in the facility. Ex-stock breeders will also be made available to the facility for other users. Furthermore, offspring born from the first pregnancy will be offered to other users within the facility or made available for use by members of the lab for example for use in optimisation of molecular analyses.

Being a pregnancy model, we study both the mother (during/after pregnancy) and her offspring (from birth to adulthood) to maximise the measurements and to integrate the outcomes observed for both mother and offspring.

Furthermore, we have an extensive tissue bank of samples that can be utilised prior to generating new animal cohorts. This tissue bank is available to both our group and external collaborators on request.

## **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.**

We will only be using mice within this project license. The majority of our methods are non-invasive and these were chosen in order to not only mimic what measurements can be (and are) performed during a human pregnancy but also to minimise any effects on the mice. Some of these methods include the measurement of blood pressure, fat and lean mass, metabolic and heart function as well as assessing placental function and the health of the unborn offspring. All of these measurements cause only very minor or no pain or lasting harm.

Only one of the methods proposed will involve surgery under anaesthesia (sedation). Surgery time is short and will be expected to last approximately 30 minutes. Once awoken the mouse will receive pain relieving medication and constant monitoring by the experimenter and animal technicians. To reduce the procedural and anaesthetic time for the female and her unborn offspring, we have devised a short protocol with a very specific aim. This will ensure that the pregnant female makes a quicker recovery.

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

For a significant number of our protocols we are able to use animals that are less sentient (able to feel things).

1. Collection of unborn offspring and their tissues.
2. Culling of pups to standardise litter size to ensure all pups get a good amount of nutrition from the mother.
3. Death under terminal anaesthesia of pregnant females following non-recovery echocardiography / ultrasound.
4. Death under terminal anaesthesia of offspring following a euglycaemic clamp.
5. Death by perfusion fixation under terminal anaesthesia. Perfusion fixation ensures that organs and tissues are preserved. This process starts by pumping a salt-containing liquid through the blood vessels, followed by a liquid fixative (to ensure that tissue structure is maintained). Tissues will then be stored to be used later.

However, for our other work it is not possible to use animals that are less sentient. As we are modelling obesity in pregnancy and aim to assess the long-term health of the offspring, we need to use animals that are sentient.

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

Based on our previous experience we have been able to refine some of the procedures to minimise harm. Surgery will be performed under aseptic conditions (set of procedures that protect the mouse from infection during surgery), anaesthesia (state of unconsciousness/sleep) and analgesia (pain relief) will be provided immediately, for as long as necessary. There will also be increased monitoring of

these animals which will include measurement of body weight and regular checks on wound healing. Any animals exceeding a certain level of weight loss will be humanely killed. Mice will also be provided with soft post-operative bedding, nesting materials, heat pads and mash food where necessary. Pain will be monitored, and pain relief provided in the form of oral medication (e.g., flavoured jelly, paste or milkshake liquid).

Once our experimental animals become pregnant, these females are often housed alone. The reason we do this is to increase the pregnancy monitoring to ensure the health of the dam and her unborn offspring. Single housing also allows us to collect individual data on the dam during pregnancy (e.g., food intake). To create an enriched environment for singly housed animals, enrichment (e.g., tubes, tunnels, houses, nesting materials) will always be provided.

We routinely acclimatise our mice to the blood pressure machine over a period of training. We will continue to do this within this license and for other settings where restraint or handling is required. A similar period of training is used for mice that will be exercised such that we start with lower speeds to allow them to become familiar with the equipment and then gradually increase the running speed over time. There is also a sponge at the back of the treadmill that encourage mice to run, rather than standing stationary. Mice will typically be put on the treadmill at the onset of the dark cycle mice to take into account their nocturnal nature. Mice that refuse to run on multiple occasions will be removed from exercise training.

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

There is a wealth of resources published on the NC3Rs webpages and this is our first port of call for guidance on best practice, in addition to following and adhering to the ARRIVE guidelines. We will also use and refer to the PREPARE guidelines when planning animal experiments. Further to this, especially for use in surgery, we will refer to guidance from the Laboratory Animal Science Association (LASA), especially LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section. (E Lilley and M. Berdoy eds.).

We also seek advice from our peers especially those who have successfully published similar studies in mice to gain from their experience.

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

We will maintain good contacts with the lead individuals (Named Persons) within our animal facility where we will be informed of any new developments or changes. We also have a 3Rs committee at the establishment and their minutes are available via our institution intranet. Our group will attend termly facility user committee meetings where new information is disseminated.

We will also aim to ensure that people working under this license are subscribed to the 3Rs newsletter from the National Centre for the 3Rs (NC3Rs). In addition to this we will continue to seek knowledge from the NC3Rs webpage (<https://nc3rs.org.uk/resource-hubs>), and 3Rs tools in-house and external resources such as the Laboratory Animal Science Association (LASA), RSPCA scientific group and Norecopa (<https://norecopa.no/databases-guidelines>).

In addition to this we are also aware of symposiums, talks, webinars and workshops all discussing the 3Rs. We will endeavour to stay on-top of advancements by attending these events as regularly as possible.