Home Office

NON-TECHNICAL SUMMARY

Physiology of inter-organ communication across the life-course

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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Physiology, Pregnancy, Placenta, Growth, Organ communication

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The goal of this project is to understand how organs "talk" to each other — not just during pregnancy, but throughout the entire life of an individual.

By uncovering how these signals work, we hope to advance our understanding of biology, discover new causes of disease, and ultimately help develop better treatments for health conditions that begin before birth and continue across the lifespan.

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?

This research explores how different parts of the body "talk" to each other — a process known as inter-organ communication. We still know very little about how cells in one organ send signals to cells in distant organs, but these signals are essential for healthy development and staying well throughout life.

Problems in these communication systems may lead to serious health conditions. By studying how organs interact, we hope to uncover new causes of disease and identify better ways to treat or even prevent them.

Why is this important?

Healthy Pregnancy and Early Development

During pregnancy, signals between organs help guide how a baby grows. When these signals are disrupted, it can cause complications like foetal growth restriction (FGR), pre-eclampsia (high blood pressure in the mother), or gestational diabetes (high blood sugar during pregnancy).

FGR affects about 1 in 10 pregnancies and is linked to a higher risk of stillbirth and health problems later in life, including learning difficulties and chronic diseases.

Interestingly, when one organ grows more slowly than others, the rest of the body sometimes “waits” to catch up — a sign that organs may coordinate with each other in ways we don’t yet understand.

Lifelong and Generational Health

Our environment — the food we eat, the air we breathe, and even the microbes we encounter — can change how our organs communicate. These changes may contribute to diseases like diabetes, heart disease, and cancer.

What's more, these effects might not stop with one person: they could influence future generations through changes to reproductive cells. But we still don't fully understand how this happens.

In short, this project will help reveal how the body's organs work together during life and how problems in this system can lead to disease. It may also help us find new treatments for pregnancy complications and long-term health issues like obesity and diabetes.

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

This research will help us learn how different parts of the body "talk" to each other — how organs send and receive signals to work together properly. We want to understand how genes and things in the environment, like diet or stress, can affect this communication.

We will share our findings with other scientists through published papers and conferences, but also with the public through media and public events.

One key goal is to understand how certain diseases start during pregnancy — like high blood pressure, diabetes in the mother, babies not growing well in the womb, or being born too early. We also want to know how conditions in the womb can affect a baby's long-term health and whether "health signals" can be passed from one generation to the next.

In the short term, we will create useful animal models to study how organs interact. This will help us explore how the placenta and the mother's health affect the baby, especially the brain as it develops.

We will also look at how signals from the sperm and egg influence the future health of the baby, right from the start of life.

In the long run, this research could help lead to new medicines that prevent or treat health problems caused by organs not working well together.

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

The main people who will benefit from this research are scientists, including those working in universities and in companies that develop medicines. We plan to publish at least 10 research papers, as well as review articles and book chapters, to help other scientists better understand how organs in the body work together.

In the long term, this work could also help doctors and healthcare professionals. By learning more about how organs communicate, we may be able to improve how we spot health problems early — especially those linked to growth and how the body uses energy, like diabetes or problems during pregnancy.

Our findings may also help other scientists start new research or even develop new treatments in the future. These new ideas might come from our team, in partnership with others, or from scientists in

different parts of the world. Either way, the work we are doing will provide a strong starting point.

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

We will share the results of this research in scientific articles and at meetings with other scientists. If we discover something that could help create new medicines, we will talk to companies who make medicines to see if they can help take it further. If we make a discovery that could lead to a useful treatment, we may apply for a patent to protect that idea.

We will make all our data available to other researchers when our work is published, so they can build on it. We also think it's important to share results that didn't work as expected, as this helps other scientists avoid repeating the same steps and saves time and resources.

Any organs or tissues collected from the animals will be stored safely and may be shared with other scientists to help with future research.

This project involves working closely with scientists in the UK and other countries, including the USA and New Zealand. We believe that working together is the best way to make progress.

We also think it's important to share what we learn with the public. In the past, we've shared our work through news stories and taken part in public events like science festivals. We will continue doing this so that everyone can learn about our research and how it could help people in the future.

Species and numbers of animals expected to be used

- Mice: 12500

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.

For our research, we need to study a type of animal that develops a placenta similar to humans, and whose genes can be easily changed so we can study how different genes affect how organs in the body work together. The mouse is the only animal that fits all these needs, which makes it the most suitable model for our work.

We will use two types of mice in this project:

- Genetically altered (GA) mice, which have small changes made to their DNA using genetic engineering. These changes help us understand the role of specific genes in how organs communicate.

- Wild-type (WT) mice, which have not had their DNA changed. These mice help us understand how things normally work in healthy development.

We will study mice at different life stages — from embryos and foetuses to newborns, young mice, and adults (both pregnant and non-pregnant, and lactating and non-lactating). This will give us a full picture of how organ communication works and changes over time.

Some of the genetically altered mice may develop health problems like poor growth, being overweight, or having trouble managing sugar levels in their blood. Others might show changes in how their organs grow, like differences in limb size or organ shape. To study these effects, we will carry out tests that measure body weight, organ size, body length, and how the mice process and store sugar and fat throughout their lives.

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

Our work is divided into two main areas: experiments on parents (before and during pregnancy) and experiments on their offspring. We will use both genetically altered (GA) mice and wild-type (WT) mice.

Parental Experiments

In these studies, we will look at how the health and environment of the mother and father — including their genes, diet, and response to stress — affect their own biology and the development of their offspring.

Some examples of what may be done:

- **Diet changes**: We will feed parents specially designed diets (e.g., high-fat food) to study how nutrition affects their metabolism and their offspring's development. These diets are not expected to cause distress, apart from the desired effect (e.g. obesity).

- **Metabolic tests**: Parents will undergo routine tests to measure how their bodies handle sugars and fats. These include fasting for short periods, receiving small doses of sugar, insulin, or fat (by injection or through a soft tube to the stomach), and taking small blood samples from a surface vein. These procedures cause brief discomfort but no lasting harm.

- **Single housing in special cages**: To measure food and water intake, energy use, and waste production, mice may live briefly (up to 4 days) in individual cages. This is done with minimal stress and includes an acclimatisation period.

- **Mild environmental stress**: Some pregnant mice may be kept in a lower oxygen environment to mimic real-life pregnancy stress. This mild stress can trigger health conditions we wish to study, especially in genetically altered mice.

- **Therapeutic testing:** Pregnant females may receive substances (e.g. growth factors, blood sugar regulators, or tiny packages called extracellular vesicles) by standard injection methods. If longer treatment is needed, a small pump will be placed under the skin under anaesthesia. This pump slowly releases the treatment over time.

- **Surgery:** Some mothers will undergo a procedure to reduce blood flow to their unborn pups — a model for pregnancy complications. This involves placing clips on the main uterine blood vessels under general anaesthesia. The surgery is carefully controlled and animals are monitored throughout.
- **Advanced imaging and tests:** Some mice will have ultrasound scans, body scans, or bone checks to monitor development and disease. These tests may involve brief restraint or contrast agents to improve image quality. In some cases, weak radioactive substances will be used to trace how nutrients pass from mother to baby. These mice will not be woken after anaesthesia and will be humanely killed to allow for tissue collection.
- **Tissue collection:** At various life stages (pregnancy, embryo, foetal, and adult), organs like the brain, liver, or placenta will be collected under anaesthesia to study how organ communication is controlled and affected by genetic or environmental factors.

Offspring Experiments

In these studies, we will examine the health and development of the offspring born from the parental experiments.

Some examples of what may be done:

- **Metabolic testing:** Offspring will have their growth, metabolism, and organ function studied using the same types of tests as their parents. This includes measuring how they use sugar and fat, how they respond to food, and how their organs develop.
- **Treatments**: Like their parents, offspring may receive treatments by injection or through a mini-pump to explore whether we can correct health problems like poor growth or diabetes.
- **Labelling and imaging:** Some offspring will receive special dyes to track bone and cell growth. These dyes are safe and used for short-term labelling, after which animals are humanely killed for organ analysis.
- **Final procedures:** In some cases, offspring will undergo a specialised test called a "clamp" to assess how their bodies use insulin. This is done under anaesthesia and the animal is not woken afterwards. Their organs will be collected for detailed analysis.

Longer-term studies will follow how the effects of diet, stress, or treatments in parents are passed on through multiple generations. This means that some studies will continue for up to three generations of mice.

Throughout the project, all procedures will be carried out with careful monitoring and efforts to minimise pain and distress. Many of the tests involve only mild or short-lived discomfort, and all surgical or invasive procedures are performed under anaesthesia with full aftercare.

A list of all procedures that may apply are listed:

- Sperm freezing – to store genetically altered strains.

- Superovulation – to collect a large number of eggs.
- In vitro fertilisation (IVF) – to create embryos from frozen sperm or for experiments.
- Embryo implantation – placing embryos into a female to produce genetically altered mice.
- Ear notching – for ID and checking genetic makeup.
- Subcutaneous, intra-peritoneal or oral dosing – to give treatments like glucose or insulin, to label cells.
- Mini-pump implantation – slow-release drug delivery under the skin.
- Blood sampling – for routine checks (e.g. liver or kidney function).
- Diet or water supplements – to test nutrition or label proteins.
- Non-invasive scans (e.g. TD-NMR, MRI, CT, DEXA, Doppler) – to assess body composition, bone development, blood flow.
- Glucose, insulin, and fat tolerance tests – to see how well the body uses energy.
- Euglycemic clamps – to test insulin function in target tissues.
- Tail cuff - to measure blood pressure.
- Placental transfer tests – to track how nutrients pass from mother to foetus.

Procedures with more than mild and short-term effects (moderate impact):

- Low oxygen exposure (hypoxia) – mimics pregnancy stress; may reduce appetite and weight.
- Uterine artery ligation – reduces blood flow to the foetus; may cause mild high blood pressure in the mother and growth restriction in the foetus.

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

The major surgical procedures in this project are of moderate severity and may cause short-term moderate pain. However, all animals will receive appropriate pain relief, and their overall condition is not expected to be significantly affected. These procedures will be performed under sterile conditions, minimising the risk of infection.

Procedures such as oral gavage, injections, and mini-pump implantation may cause transient discomfort, but not lasting harm. On rare occasions, mild infection or inflammation may occur at injection or implantation sites.

Some procedures, such as blood pressure monitoring, imaging, or placing animals in low-oxygen chambers or metabolic cages, may cause stress. This will be reduced through brief adaptation periods.

While single housing may also induce stress, it is necessary for accurate metabolic data and will be kept short, with enrichment (e.g., nesting materials and shelters) provided to reduce distress.

Mild and short-term procedures include blood sampling, imaging (which may involve brief restraint), injections of substances (including labelling agents), and temporary housing in metabolic cages.

Health issues due to genetic manipulation are expected to be minimal or mild in most cases. The main effects may include growth abnormalities, impaired metabolism (e.g. insulin resistance or high blood sugar), and, in a small number of cases, developmental defects during foetal life such as foetal loss, prematurity, or abnormal organ growth.

Some mice may become pre-diabetic, diabetic, or obese due to dietary or genetic changes, or develop high blood pressure. During insulin tolerance tests, some may experience low blood sugar (hypoglycaemia), which will be promptly treated with sugar injections. Glucose and insulin tolerance tests may require fasting for up to 16 hours (6 hours for pregnant females), with weight loss monitored closely. Mice losing more than 20% body weight will be re-fed immediately and excluded from further testing.

Signs of ill health — such as pilo-erection (bristled fur), subdued behaviour, appetite or weight loss (>15%), inactivity, shortness of breath, or diarrhoea — are expected to be rare. Animals are monitored carefully, and any showing signs of illness will be treated or humanely euthanised, following strict welfare criteria.

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)?

Based on similar previous work and retrospective assessment of current PPL we expect severities to be 50% sub-threshold (i.e. expected to experience effects below the threshold for noticeable suffering), 43% mild, and 7% moderate, which applies to both GA and WT animal types.

What will happen to animals used in this project?

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

We need to use animals because our research focuses on how organs interact in a living body throughout life — something that cannot be fully studied in cells or tissue samples alone. Mice are the most suitable model for this work. Over the past few decades, scientists have developed genetic tools that allow us to study the role of almost every gene in mice. Mice also share many important biological features with humans, making them valuable for understanding human health and disease. To turn basic scientific discoveries into real-world treatments, it's important to test ideas in living systems. The mouse models we are developing will allow us to do this in a controlled, pre-clinical setting.

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

Some parts of our research can be carried out using cells in a dish or small lab-grown versions of organs, called organoids. Organoids are simplified 3D models of real organs that help us study certain aspects of how cells work. We also use organ-on-a-chip technology — miniature systems that recreate some features of real organs using tiny fluid-filled channels to keep cells alive and functioning. We use these methods whenever appropriate. However, they cannot fully reproduce the complex communication that happens between different organs in a living body. For example, how the placenta, brain, and liver "talk" to each other during pregnancy can only be studied in a whole animal.

Observing and carefully manipulating these interactions in live animals is essential for meeting our research goals. Where possible, we will combine animal studies with follow-up experiments in cell cultures and organoids to explore specific biological pathways in more detail.

Why were they not suitable?

To fully understand how organs in the body communicate with each other, we need to study these interactions in a living organism. Cells in the body send signals to other cells — sometimes across long distances, like from fat tissue to the brain — using the bloodstream. This kind of complex, multi-organ communication cannot be studied in isolated cells or lab-grown mini-organs (or organoids).

To answer the questions in our research, we need to use live animals to measure things like nutrient transfer from mother to baby, blood flow to the womb, and how the body responds to diet changes or treatments for gene-related diseases. These studies are only possible in whole animals.

However, we will also run as many experiments as possible using cells and organoids to reduce the number of animals used. These lab-based studies will help us understand what goes wrong inside specific cells when certain genes are altered. For example, we will use advanced genetic tools to switch off specific genes using special molecules called RNAs that guide “molecular scissors” to cut out target genes. We will also isolate cells directly from mouse tissues — known as primary cells — for more detailed study. In addition, we will use human samples to help link our mouse findings to human health. These include donated samples from hospitals such as maternal blood, placental tissue, fat, and pregnancy imaging data.

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 base the number of animals needed on two decades of experience and data from similar studies. For new experiments, we use published data or carry out small pilot studies to measure how variable the results are. This helps us calculate the minimum number of animals needed to detect meaningful differences between experimental and control groups.

For most physiological tests — such as glucose tolerance (how the body clears excess sugar from the blood) or placental transfer (how well nutrients pass from mother to baby) — we typically need around 8 to 12 animals per treatment group.

For growth studies, we use advanced statistical models developed using our own historical data. These models help us track growth changes accurately while keeping animal numbers as low as possible.

The number of mice that need to be bred also depends on the type of genetic change. Some genetic alterations can affect a female's fertility or egg quality, meaning more animals must be bred to obtain the required number of experimental mice.

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

We use careful experimental design to reduce animal use wherever possible. This includes:

a) **Power calculations** – which help us estimate the smallest number of animals needed to get reliable results, and

b) **Optimised breeding strategies** – which aim to reduce the number of animals that do not carry the genetic change we are studying.

Our sample size estimates are also checked using tools recommended by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), such as the Experimental Design Assistant (https://nc3rs.org.uk/experimental-design-assistant-eda).

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

We are committed to reducing animal use wherever possible. To achieve this, we use ex-vivo methods (studying organs or cells taken from animals) and in-vitro methods (growing cells in a dish). In previous work, we developed models using placental and pancreatic cells and have used established cell lines to reduce or replace the need for live animals in certain parts of our research. In this project, we will continue to use these models and add newer methods, such as mini-organs (organoids), which allow us to study organ function outside the body. Although we cannot fully replace live animals for studying

whole-body processes like communication between organs, in-vitro and ex-vivo experiments help us understand specific aspects in detail and reduce the number of animals required.

We minimise animal use by collecting as much data as possible from each individual mouse, including multiple measurements and tissue samples. At post-mortem, we routinely collect and store extra tissues — building a ‘tissue bank’ that allows us and others to conduct future studies without needing new animals. We share tissues, cells, and experimental results with collaborators, helping reduce the number of animals needed across multiple projects.

Every foetus or neonate provides several organs, which can be studied in relation to specific conditions (e.g. growth) and used by different researchers, increasing the value of each animal used. We also reduce control animal use by sharing wild-type mice across project licences and participating in local schemes (e.g. Strain Enquiry lists) that promote sharing of surplus animals and tissues.

We use non-invasive techniques whenever possible, allowing repeated measurements in the same animals. Moreover, we are refining methods to study the effect of high levels of hormonal stress in developing eggs in a dish — exposing them directly to stress hormones without subjecting live females to highly stressful situations (e.g. predator scent or restraint), which would be otherwise required to obtain similar rises in stress hormones levels. This helps reduce both animal numbers and suffering.

Before starting animal experiments, we often perform pilot studies in cells to refine our approach. Our experimental design and reporting follow best-practice guidelines from the NC3Rs, including PREPARE (planning) and ARRIVE (reporting).

We prepare detailed study plans outlining the objectives, methods, number of animals, and analysis plans. Data is collected blindly — researchers don’t know which animals belong to which group (e.g. treated vs control) until after the data is collected. Whenever possible, we also use randomisation (i.e. arranging a set of animals in a random order) to assign animals to groups, helping to reduce bias and ensure robust results.

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 use mice at all stages of development, including both genetically altered (GA) and wild-type (WT) animals. We will use both standard and state-of-the-art methods to produce GA animals and to study how organs communicate during pregnancy, lactation, and after birth. Most procedures in this project are classed as mild and cause only brief, short-term discomfort. In fact, over 50% of the animals used are expected to experience effects below the threshold for noticeable suffering.

We implement a range of refinements to reduce pain, distress, and overall harm at every stage of the experimental process. These include:

1. Analgesia and Anaesthesia

Oral analgesia: Where possible, we will replace injectable analgesics with oral formulations to reduce handling stress and injection-related discomfort. For example, carprofen will be administered in palatable jelly to avoid subcutaneous injection.

Pre-emptive and multimodal pain relief: Analgesics will be administered before and after procedures likely to cause discomfort (e.g., embryo transfers, surgical interventions), combining agents where needed to cover multiple pain pathways.

Refined anaesthetic protocols: We will use the least invasive and shortest-acting agents appropriate for each procedure, and tailor recovery environments to ensure comfort and rapid return to normal behaviour.

2. Non-Invasive and Minimally Invasive Techniques

Non-invasive imaging (e.g., TD-NMR, ultrasound) is used wherever feasible to reduce the need for surgical sampling and longitudinally monitor animals, also reducing total numbers used.

Minimally invasive blood sampling and non-terminal sampling methods are prioritised for metabolic assessments.

3. Housing and Handling

Habituation to procedures: Animals will be pre-trained for procedures such as restraint for blood pressure measurement to minimise stress responses.

Refined single housing: When single housing is necessary (e.g., metabolic cages, pregnancy tracking), animals will be acclimatised in enriched environments, and duration will be minimised.

Enrichment: All animals will receive species-appropriate environmental enrichment to promote natural behaviours and reduce anxiety.

4. Early Humane Endpoints and Monitoring

Animals will be closely monitored with **structured welfare scoring** to identify early signs of pain, distress, or illness. Any animal showing adverse effects will be promptly treated or humanely euthanised in line with pre-defined humane endpoints.

Pregnant dams and offspring will receive additional checks to monitor for pregnancy-related complications or abnormal development.

5. Replacement and Reduction

Use of in vitro models (e.g., placental organoids, primary cell cultures) will be prioritised for mechanistic studies where feasible.

Longitudinal study designs allow repeated measurements in the same animal, reducing total numbers required.

Some of the new genetic changes we introduce may result in developmental issues, such as foetal loss, anaemia, or brain malformations. These outcomes are difficult to predict in advance. In such cases, we will study the early disease mechanisms before animals show signs of illness. Once tissues are collected, any further studies will be done at early stages of disease, to avoid pain or suffering. We are also working to identify early cellular markers of disease, so we can intervene before animals show visible symptoms.

For studies on the effects of stress before conception, we collect eggs from humanely killed mice and expose them to stress hormones in the dish — we could not naturally achieve these high levels of stress hormones in live mice without causing significant stress (e.g. restraint, exposure to a predator).

By integrating these refinements throughout the protocol, we aim to uphold the highest welfare standards and ensure the scientific validity and reproducibility of our data.

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

We have considered using animals that are less able to feel pain or distress. However, because our research involves modelling human diseases — including pregnancy complications — we must use a placental mammal, as only these species share the necessary biological features with humans. All placental mammals are sentient to some degree. To meet our scientific goals, we also need a species that can be easily genetically modified. Among the placental mammals that meet these requirements, the mouse is the least sentient and is therefore the most appropriate choice for this project. Many of our experiments focus on embryos and mid-gestation foetuses, which are not thought to experience pain at those stages.

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

We are committed to refining our procedures to minimise any potential harm or distress to the animals. The following measures will be in place throughout the project:

- **Group housing** will be used wherever possible to reduce stress and abnormal behaviours. Single housing will be kept to a minimum and only used when necessary (e.g. for specific measurements).
- **Environmental enrichment (EE)** will be provided, including igloos, running wheels, fun tunnels, and shelters. These help reduce anxiety and support natural behaviours. Enrichment will be compatible with procedures, including gentle handling techniques (e.g. handling tunnels) to minimise stress during tasks like injections.
- **Pain management will be a priority.** Animals undergoing procedures with potential for pain will receive appropriate analgesia and post-operative care.

- **Acclimatisation training** will be used for animals involved in specific tests such as blood pressure monitoring (tail cuff), metabolic cage housing, or exposure to low-oxygen environments. Training helps animals adapt to these situations, reducing stress and variability in results.
- **Minimal transportation** will reduce disruption. Most animals will be bred and housed in a single facility. If animals are moved, they will be given time to acclimatise before starting experimental procedures.
- **Monitoring and scoring systems** will be used to track animal welfare during procedures. These allow early identification of discomfort or health concerns, enabling timely interventions.
- **Nutritional support** will be provided for animals expected to lose weight (e.g. during certain treatments). Palatable, nutritionally enriched food supplements will be given throughout the procedure period.
- **Refinements in hypoxia models:** We will use 13% oxygen instead of 10% for low-oxygen experiments where possible. This level causes fewer adverse effects (e.g. only a slight reduction in litter size), while still allowing meaningful scientific outcomes.
- **Embryonic endpoint refinement:** In limb growth experiments, embryos will be humanely killed at different stages of gestation to assess developmental abnormalities before allowing full-term pregnancies, reducing potential suffering.

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

Laboratory Animal Science Association (LASA) guiding principles documents of aseptic technique (https://www.ubs.admin.cam.ac.uk/files/lasa_aseptic_surg.pdf)

ARRIVE (Animal Research: Reporting of In Vivo Experiment) guidelines for preparing papers for publication (https://www.nc3rs.org.uk/arrive-guidelines)

PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines for planning our experiments (15 topics including formulation of the study, dialogue between scientists and the animal facility, and methods) (https://www.ncbi.nlm.nih.gov/pubmed/28771074).

EDA (Experimental Design Assistant) - https://nc3rs.org.uk/our-portfolio/experimental-design-assistant-eda- a free online tool from the NC3Rs, designed to guide researchers through the design of their experiments, helping to ensure that they use the minimum number of animals consistent with their scientific objectives, methods to reduce subjective bias, and appropriate statistical analysis.

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

To be informed about latest advances we will primarily use the National Centre for the Replacement and Reduction of Animals in Research (NC3R) website (https://www.nc3rs.org.uk). It provides an

extensive library of 3Rs guidelines, resources, practical information and themed hubs. It also provides links to publications, other online resources, and video and training materials.

Our establishments intranet provides a portal to numerous sources of information, with a few listed below:

(AALAS) American Association for Laboratory Animals Science

(FELASA) Federation of European Laboratory Animal Science Associations

(ICLAS) International Council for Laboratory Animal Sciences

(InterNICHE) International Network for Humane Education

https://www.nc3rs.org.uk/welfare-assessment

http://enrichmentrecord.com/

https://science.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/rodentwelfaregroup

Implementation of the advances will be defined on a case-by-case basis and will be informed by the latest NC3R recommendations.

Importantly, we have access to Named Information Officers at our establishment, which can provide invaluable help with 3Rs information and resources.