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NON-TECHNICAL SUMMARY

Lactation, Nutrition, and Development: Molecular Pathways Linking Early Life to Health Trajectories

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Genes, Lactation, Nutrition, Metabolism, Lifelong health

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

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?

We aim to understand the function and regulation of genes that are important for early-life nutrition, animal body function and development throughout the life-course.

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?

Our study has important implications for understanding health and well-being across the lifespan.

Lactation is a natural process where the mother produces and releases milk from the mammary glands. It is a complex biological process, regulated by multifaceted mechanisms that ensure the proper transfer of nutrients and immune factors (protecting the offspring from diseases), from mother to offspring.

Early life nutrition plays a critical role in shaping long-term health outcomes, influencing growth, metabolic function, and immune development. Disruptions in these processes are linked to various health issues, including obesity, diabetes, and immune-related diseases (e.g. allergies, asthma). By investigating the mechanisms that regulate lactation and the impact of early nutrition, we can better understand how to promote optimal health from infancy through adulthood.

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

We will generate new knowledge about the mechanisms that regulate lactation and its influence on lifelong health. This includes understanding how early nutrition impacts growth, metabolic health, and immune function, as well as identifying factors that contribute to lactation disorders (problems occurring when the mother experiences difficulties making or releasing milk to feed her baby). Our research will also explore how maternal organs, such as the brain, mammary glands, and endocrine system (a group of glands that make up hormones serving as messengers to control body functions such as sleep, appetite), communicate to regulate milk production and composition. Disruptions in these communication pathways can contribute to lactation issues like low milk supply, mastitis (swelling in the breast usually caused by infection), and imbalances in milk nutrients, affecting both maternal and infant health.

Additionally, we aim to uncover how genetic and environmental factors influence these regulatory networks, potentially leading to long-term health consequences for mother and child. This understanding could pave the way for early diagnostic tools, targeted interventions, and novel treatments for lactation-related disorders.

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Our findings will be shared through open-access publications and made available in public data repositories, supporting further research and facilitating the translation of discoveries into clinical and public health practices.

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

Our research on lactation will deepen our understanding of the biological mechanisms that regulate milk production, composition, and maternal organ communication. This knowledge will improve the detection and management of lactation disorders, such as low milk supply and mastitis, benefiting both mothers and infants in the short term by supporting successful breastfeeding and reducing health complications.

Our studies on early-life nutrition through breastfeeding will help unravel the mechanisms shaping long-term health. This work will also highlight how disruptions in lactation may contribute to developmental and metabolic disorders (such as obesity and diabetes), guiding more effective nutritional interventions during infancy.

Additionally, by exploring how maternal organs communicate during pregnancy and lactation, we aim to identify pathways which will lay the foundation to support the development of treatments for mothers facing breastfeeding challenges and inform public health policies to promote maternal and child health.

Our findings will also contribute to understanding how environmental and genetic factors affect lactation and may influence offspring health across generations. This long-term perspective could pave the way for preventative strategies that promote healthier outcomes from infancy through adulthood.

Ultimately, our research will benefit healthcare providers, policymakers, and families by providing evidence-based insights to optimise maternal and infant health.

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

Our research is highly collaborative and we will actively seek to contribute my expertise, technology and materials, positive and negative data to others and are often sought out by colleagues who wish to collaborate with us. We have a good track record in this, as evident from the publications that we co-author with others.

We will continue to establish and participate in national and international scientific organisations to maximise the impact of our research and add value altogether. We will continue to present our work prior to publication at conferences, seminars and lectures, including unsuccessful approaches.

We are obliged by the funding agency to publish our studies only in peer-reviewed open access journals.

Species and numbers of animals expected to be used

- Mice: 25000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are used to investigate aspects of our studies on lactation, nutrition, and lifelong health. These animals are ideal models due to their well-documented genetic profile and widespread use in scientific research, enabling reliable data comparisons across studies. Furthermore, established, non-harmful experimental procedures allow for precise manipulation of genes or their regulation, facilitating the exploration of nutritional and developmental impacts across the lifespan. Many of the genes involved in lactation, metabolism, and growth in mice are also present in humans, making findings highly translatable.

To understand how nutrition and lactation influence health outcomes throughout life, it is essential to study mice across all life stages: From early development and weaning through adulthood and ageing. This comprehensive approach provides insight into critical windows of nutritional influence and their long-term effects on health and disease susceptibility.

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

For the vast majority of our work, animals will be mated, and biological samples will be collected post-mortem at various life stages, including pregnancy and lactation, to understand the impacts of nutrition and lactation on lifelong health.

To investigate metabolic processes, animals may be provided with an altered diet to assess nutritional effects. Additionally, they may receive substances such as glucose or insulin (sugar and the corresponding hormone control its blood levels) via an intraperitoneal injection (into the abdominal cavity) , to measure their response to feeding, followed by blood sampling, typically up to six blood samples over four hours collected from the tail vein. In some cases, mice will be housed in metabolic cages (cages with sensors tracking their food and drink consumption, activity and energy usage) for around 72 hours to monitor detailed metabolic activity, including food intake, energy expenditure, and waste production. These cages are available at a nearby facility.

For the study of lactation and milk composition, the mother will be separated from her pups for up to four hours to allow milk accumulation in the mammary glands. Mothers will then be anaesthetised and administered a hormone to stimulate milk production. Gentle massage of the mammary gland and nipple will be used to collect milk samples with a pipette or using a pump specifically designed for mice. Afterwards, mothers are returned to their pups, who remain in the nest undisturbed, ensuring minimal stress and natural behavioural recovery.

To explore maternal inter-organ communication during lactation and its influence on nutrition and health, mice will be administered substances, for example biotin (A vitamin used for labelling), typically through their diet or via injection, for example intraperitoneal (into the abdominal cavity), subcutaneous

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(beneath the skin) or intravenous (into the vein). Biotin is non-toxic and has no adverse effects on pregnancy or lactation, making it a safe choice for studying nutrient signalling pathways.

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

The vast majority of animals will undergo well-established procedures and will experience no adverse effects.

When new mouse lines are created or new crosses are bred we do not know the effects these will have on the offspring. Occasionally, these new lines may generate offspring with delayed developmental growth and the offspring may be born with a lower birth weight compared to their wild-type litter mates. Sometimes these mice with a lower birth weight regain a normal weight during adulthood.

During the project, any animal that has been through a procedure will be monitored for any adverse effects, examples include changes in locomotion, weighing , provision of heat mats and post operative bedding. When appropriate, pain management such as the use of medicated palatable substances for voluntary treatment, e.g. flavoured jelly will be given.

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: Sub-threshold - up to 60%

Mild - up to 25%

Moderate - up to 15%

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

To understand how lactation and early nutrition affect lifelong health, it is essential to study these processes in a living organism where all body systems interact naturally. Mice are the most suitable

model for this research because their lactation and mammary gland development are similar to those in humans, and their genetics are well-mapped and easily manipulated. This allows us to study specific biological pathways during pregnancy, lactation, and early development, which is not possible with simpler models like cell cultures.

Lab-grown cells can only represent a small part of an organ and do not mimic the complex interactions between tissues, organs, and the offspring during nursing. Similarly, alternative models cannot replicate the mother-offspring relationship or the communication between organs that is crucial for understanding nutrition's impact on growth and health.

By studying mice, we can observe how nutrition during critical stages influences development, metabolism, and long-term health, insights that are vital for understanding human health and disease prevention.

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

When possible, we use non-animal alternatives before moving on to animal models.

Such alternatives include lab-grown cell experiments, which allow us to answer simple questions in a non-organismal context. We perform initial experiments in cells to test some of our hypotheses and choose the best candidate genes and pathways to focus on before moving on to mouse experiments. This way, we can reduce the total number of animals we use.

We use both our own and public databases, reanalyse existing data when possible, and use computer modelling and bioinformatics.

Why were they not suitable?

Very often, the non-animal alternatives are not suitable. We cannot conduct experiments that address the health of an entire animal in the dish, which lacks the complexity and the interactions occurring in a physiological context.

When studying maternal-offspring interactions, there are no alternative non-animal methods that are sophisticated enough to address behavioural, hormonal, genetic and physiological questions which are very important aspects of our research programme.

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?

Our estimates are based on over 16 years of experience in mouse genetics and more than 7 years of focused research in this area. To ensure accuracy and minimise unnecessary use of animals, we calculate required numbers using robust statistical tools, drawing on data from previous experiments, pilot studies in lab-grown cells, and small-scale preliminary animal work.

This research focuses on understanding how traits and signals are passed down from mothers and fathers. To do this, we need to compare maternal and paternal inheritance separately, which means setting up breeding pairs for both maternal and paternal lines, effectively doubling the number of animals needed for each experiment.

Furthermore, the biological questions we are addressing, particularly those related to pregnancy and mammary gland development, can only be studied in female mice. As roughly half of the offspring in each litter are male and therefore not suitable for these specific experiments, only about 50% of the animals born can be used.

In addition, we often work with specific genetic mutations that are only present in a quarter (25%) of the offspring due to the nature of the breeding strategy. Of those, only female mice are relevant to our studies. This means that ultimately, only around 25% of the animals in each litter are suitable for use in our experiments.

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

We use online tools such as NC3Rs EDA https://www.nc3rs.org.uk/experimental-design-assistant-eda and PREPARE guidelines https://norecopa.no/PREPARE, and NC3Rs website https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction .

Blinding and randomisation is being used when possible, ensuring reproducibility and subsequenctly reducing the number of animals being used. https://nc3rs.org.uk/3rs-resources/using-maskingblinding-vivo-experiments

Additionally, we consult collaborators or experts when we need to design new experiments in which we need to acquire additional expertise.

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

1. Efficient breeding

   We work closely with animal technicians in the facility, who will advise us if breeding pairs are not producing any litters or producing fewer litters. Furthermore, all breeding information is recorded in our online colony management system, where we record all animals and their procedures. Therefore we will notice immediately if breeders have problems.

   This breeding strategy is necessary to generate all the required genotypes to maintain the genetic lines we study. To ensure responsible use of all animals, we have developed a coordinated internal system that allows other researchers in the lab to make use of animals not

required for our specific studies, for example, for breeding purposes, pilot studies, or tissue collection. This approach helps maximise the scientific value of every animal born.

2. Sharing of animals, tissue samples, and cell lines. We communicate regularly to share animals, tissue samples, and cell lines. We use shared files to summarise and share the information of stock tissue samples and cell lines. All animal information has been recorded in colony management system, therefore animal users can easily check which animals are available.

   We share animals, tissues and cell lines with our collaborators worldwide. This means that others do not need to generate new mutants or samples, minimising animal numbers.

3. Preservation of mouse strains: We freeze sperm or preimplantation embryos from mouse strains that are not planning to use at least for a couple of years.

4. Pilot studies

   Where possible, we conduct initial studies on cells in a dish or with a small number of animals to determine whether experiments are then required in larger numbers. Occasionally our scientific questions can be answered by laboratory based experiments alone.

   We optimise protocols with small-scale experiments with wild-type samples before conducting experiments using mutants.

5. Rederivation of mouse lines - we recently rederived all our mouse lines, moving them into a clean facility. This resulted in increased breeding efficiency and a reduction in the number of mice being used.

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.

For our lactation research, we use mice as the primary animal model. Mice are ideal for this type of study because their biology closely mirrors key aspects of human lactation, and their genetic makeup is well-understood and easy to manipulate. This allows us to study specific genes involved in milk production and maternal health without affecting other organs or the offspring.

We use tissue-specific technology to introduce targeted changes in the mouse genome. This method is highly efficient and precise, enabling the use of fewer animals and a shorter time compared to traditional breeding techniques with general genetic manipulation. Because the genetic changes are limited to a single tissue, the mice experience minimal impact on their overall health and behaviour.

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This technique also allows us to obtain accurate results using fewer animals and in a shorter time than traditional breeding methods, further reducing the number of animals involved in research.

Furthermore, the controlled breeding and well-established reproductive protocols for mice reduce variability, which helps us achieve clear results with fewer experiments, limiting the animals' exposure to procedures. All animals are housed in enriched environments and monitored closely to ensure their wellbeing.

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

Our research focuses on understanding lactation and how it influences health and development in mammals. Since lactation is a complex biological process unique to mammals, simpler or less sentient species cannot model it effectively. Mice are the most appropriate choice because their lactation processes closely resemble those of humans, allowing us to study gene function and maternal-offspring interactions in a meaningful way.

Additionally, we need to observe these processes across different life stages—from pregnancy and lactation through early development—to understand their full impact. Studying only immature life stages would not provide insights into how early nutritional experiences affect long-term health. Therefore, using mice is essential to achieve our research goals.

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

We will use well established protocols and will carry out pilot studies before starting procedures. Procedures will be discussed with experienced collaborators to minimise welfare costs for the animals.

Any animal that has been through a procedure will continuously be monitored at regular intervals examples include observing changes in locomotion, weighing , heat mats and providing post operative bedding. When appropriate, pain management such as the use of medicated palatable substances for voluntary treatment, e.g. flavoured jelly will be given. Needles are being used once, to prevent pain, reduce the risk of infection and maintain high standards of care.

Mice are handled in a non-aversive manner, typically group housed with enrichments such as chew blocks, tunnels, mouse houses and nesting material.

Where substances are supplemented in drinking water, e.g. we may use sweeteners if possible, to support palatability. When using substances for gene alteration, the dose required is low and does not affect pregnancy outcomes or lactation. Guidance is available for specific substances from the University Biomedical Services.

When performing vaginal swabbing, we use a refined way to sample vaginal cells, without requiring insertion of a pipette into the vagina, and while the animal is in a tunnel rather than restrained.

If possible, for any new mouse lines, inducible constructs (genetic tools allowing to switch on/off genes at a certain time) will be used so that mice only display a phenotype once the deletion is induced. We

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will refine the humane end point for new mouse lines and will seek advice from the Named Veterinary Surgeon and the Home Office Inspector if needed.

In this licence, we implemented the use of a milk pump as an alternative for manual expression. This allows the collection of substantially more milk and mimics the pup better.

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

To ensure our lactation research is conducted in the most refined and ethical way, we follow established best practice guidelines. This includes adherence to the ARRIVE guidelines, which help us design robust and well-structured studies. These guidelines cover critical aspects such as study design, sample size calculation, randomisation, blinding where possible, and the use of appropriate statistical methods to ensure reliable and ethical research outcomes.

We also follow the PREPARE guidelines, which emphasise careful planning before any animal studies begin. This includes reviewing literature, ethical considerations, study design, and collaborating with animal facilities to ensure optimal housing, health monitoring, and humane endpoints.

My extensive experience in conducting mouse studies, alongside published protocols in mammary gland, liver, and brain research, further supports the refinement of our experimental approaches. We consistently review and update our methods based on the latest published protocols to maintain the highest standards of animal welfare and scientific integrity.

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

We will regularly discuss with the Named Persons at our institution on the best practices and new approaches in the 3Rs, including using the NC3Rs website pages https://nc3rs.org.uk/resource-hubs. We have subscribed to the NC3Rs e-newsletter to receive information on the latest 3Rs developments as well as information on NC3Rs events and workshops, which we can attend. We consider the latest practical guidance from Laboratory Animal Science Association (LASA), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA). Additionally, we use external resources such as Jackson labs database https://resources.jax.org/. Lastly, we will maintain contact with our local NC3Rs Regional Programme Manager for advice on implementing advances in the 3Rs.

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