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NON-TECHNICAL SUMMARY

The evolution of the placenta across vertebrates

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

placenta, pregnancy, evolution, placenta development, pregnancy immunology

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Poeciliidae	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

We aim to understand how new organs originate and how they subsequently change in form and function across species using the placenta as a model. The placenta has evolved multiple times independently across vertebrates and we study its biology, development, and evolution in mammals and fish.

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?

The placenta is responsible for transferring nutrients, oxygen, waste, and hormones between the mother and her embryo(s). Without the placenta, none of us would be here. Despite its crucial role, the placenta is one of the most poorly understood organs, and as a consequence, so are many pregnancy complications. An example is pre-eclampsia, a condition associated with maternal high blood pressure that can impact as many as 1 in 12 pregnancies and is a leading cause of maternal ill-health. This project aims to bring much-needed insight into the placenta, how it develops across different mammals, and how it evolves across species. One of our focuses is on one of the most fascinating aspects of pregnancy - the mother’s tolerance to the direct contact between her own cells and those of her foetus. We study how maternal immune systems have evolved different solutions in different mammals (including in humans) to deal with the challenges of the foetus on the mother’s immune system.

The placenta is not, however, exclusive to mammals. Placentas have evolved more than 100 times independently across different vertebrates (for example, in some lizards, snakes, amphibians and fish). This makes the placenta an exceptional organ in which to study how organs originate and how they subsequently change in form and function across species. One family of small neotropical fishes (Poeciliidae) is exceptional from this point of view. Within this fish family (which includes guppies and mollies), placentas have evolved independently multiple times. We study this unique fish family to identify general principles (or rules) guiding the evolution of new organs.

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

There will be three main overarching outputs. One output will be a better understanding of the evolution of the mammalian placenta from a maternal perspective. Across mammals, maternal immune systems have evolved a variety of solutions to deal with the challenges of pregnancy. Identifying and understanding these solutions will shed new light on the evolution of the placenta. It will also contribute to our understanding of pregnancy complications, and it can potentially teach us about the immune rejection of transplants.

A second main output will be a description of how novel complex organs can evolve from scratch. Among Poeciliids, a placenta has evolved independently multiple times, and although sharing many features, the placentas from different species also show distinct morphologies. We aim to identify general principles underlying the formation of a new organ.

A third main output will be the identification of animal models that best mimic different aspects of the human placenta. Because the placentas of different species differ greatly, there is not a single best species that can serve as a proxy for the human placenta. By understanding the similarities and differences among placentas of different mammals, we will be able to identify the animal models that best resemble the human placenta.

We will share these outputs through publications in scientific journals and communications in scientific conferences and the press to quickly and freely disseminate our results to the general public and the scientific community.

We will also create a user-friendly (and freely available) database that compares placenta development across species. This database will allow the scientific community to access our data quickly and easily, maximizing its impact. It will also help in the choice of species and developmental stages for studying specific aspects of human placenta development.

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

The primary beneficiaries of these outputs will be other research groups that study the human placenta (in health and disease) and that of other species. We expect our novel findings to start being disseminated in scientific publications and conference communications two thirds into this project and continue for the ensuing years.

An output of this project will be a database, which will be available to all, describing (at the molecular level) placenta development across many species. These data will eliminate the need for smaller, targeted studies of individual species and/or developmental stages and serve as a resource for the scientific community. In the long run, it will reduce the number of animals used for these purposes. We anticipate making most of these data available before the completion of this project.

By comparing placenta development across multiple species, we will create a resource that will aid in choosing the most appropriate species to study different features of the human placenta. We anticipate that this will have a significant impact on the placenta biology community.

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

We will create a database to make our data easily accessible to all researchers. We will produce the data in an unbiased and systematic manner to serve as a community resource. We expect our database to eliminate the need for smaller, targeted studies of individual species and developmental stages and hence, the number of animals involved.

We will disseminate the results from our work in multiple scientific publications. These peer-reviewed publications will be open access, that is, freely available to all. We will also disseminate our results through scientific conferences, national and international. To make our work accessible to the general

public, we will write summaries for a lay audience and disseminate them in suitable outlets (general press).

This research involves collaborations with research groups from the UK, Europe, and the USA.

Species and numbers of animals expected to be used

- Mice: 4800
- Other fish: No answer provided

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.

The development of the placenta depends on an active collaboration between the mother and the embryo. There are currently no *in vitro* systems that can model this process properly. Our mammalian work will be primarily based on a collection of frozen tissues (placentas) obtained before the start of this project from several mammals (including humans, marmosets, mice, rats, rabbits, guinea pigs, horses, sheep, bats, and opossums). We acquired these frozen samples through multiple national and international collaborations. However, to study specific aspects of placenta development and function, we must work with genetically altered mice. We will work with two groups of genetically altered mice. One group of mice have modifications that allow us to visualize under the microscope the cells where specific genes are active, which is key to understanding placenta development. The other group comprises mice with genetic modifications that may impact the placenta, such as the maternal immune response to pregnancy. We are asking for permission to breed these mice in our animal facility to study their placentas. The placenta is an embryonic tissue that is only present before birth. Therefore, our work will focus on embryos and fetuses of both sexes and pregnant female adults.

The family of fish Poeciliidae (that includes guppies) is an exceptional model to study how placentas evolve from scratch because, within this family, placentas evolved independently multiple times. This means that we can study closely related species with and without a placenta, something not possible in mammals. Like in mammals, the fish placenta is an active collaboration between the mother and embryo and only exists before birth. Hence, our fish work will also be based on embryos of both sexes and adult pregnant females.

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

Mice:

- We are asking to breed genetically altered mice so that we can collect placental tissues from pregnant females. The tissues will only be collected after the animals are humanely killed using Schedule 1 approved methods.

- Some mice will have genetic modifications that need to be activated by giving the pregnant female an activating substance. We will only use activating substances that do not produce adverse effects beyond their administration's mild and transient discomfort (e.g. oral gavage) or that cause adverse effects that can be counteracted by administrating other substances. For example, the adverse effects of tamoxifen, an activating substance, can be counteracted by the administration of progesterone. The activating substances will only be administered once per individual. We will collect placental tissues after the pregnant females are humanely killed using Schedule 1 approved methods.

Fish:

- We will humanely kill fish using approved methods. In addition to collecting placentas, we will also collect other organs/tissues (e.g. brain, ovary) to create a tissue biobank that we and others can use for future work, thus eliminating the need for further animal use.
- Following veterinary advice, we may use hormones to induce some fish species to mate in case of low performance. The hormones will be added to the water in concentrations that will not adversely affect the fish. We will only use hormones if all other methods available fail (e.g. changes in nutrition, water temperature, environment enrichment) and if there are no known adverse effects associated with these hormones.

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

- Most genetic alterations in the mice that we will breed and maintain either have no impact on the animals or only have mild effects that do not impact their life quality in a laboratory setting.
- Some genetic alterations may be associated with some embryo loss during early to mid-gestation or with difficulties in embryo implantation in the uterus. The latter is not expected to lead to adverse effects on the pregnant female, and the embryos are lost at a very early stage of development.
- The administration of activating substances produces mild and transient discomfort. We will only use activating substances that do not have significant adverse effects or whose adverse effects can be alleviated using other substances (e.g. progesterone in the case of tamoxifen).
- No adverse effects are expected for the fish. We will only use hormones if they do not have side effects and are approved by the veterinarian.

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)?

The severity of all protocols is set at mild, but the expected severity for the fish is sub-threshold.

What will happen to animals at the end of this project?

- Killed
- Used in other projects
- Kept alive

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?

The development of the placenta is a cooperative process between the embryo (from which the placenta originates) and the mother. There is a special tissue that forms during pregnancy in the uterus, called decidua, which works together with the placenta to provide the developing embryo with the nutrients and everything else it needs to grow. It is the placenta and decidua combined that is the functional unit that allows animals to grow in their mother's womb. Pregnancy is also a process that differs in many essential details between species. For both these reasons, we cannot study placenta development using *in vitro* (cell culture) systems. We also know so little about this system that we do not know how to approximate *in silico* these complex interactions between the mother and the embryo, and so no realistic *in silico* models exist yet (but we hope our research will ultimately help make these models possible). The placenta needs to be studied in its native complex context, and we can only understand the diversity of placenta forms and functions between species by studying those species.

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

Because the development of the placenta depends on both the embryo and the mother and the complex interactions between them, it cannot be understood outside its native (animal) context. The existing *in vitro* models of embryo development do not include the placenta. Even if they did it, the critical maternal contribution, the decidua, would still be missing. However, we have tried to minimise as much as possible the use of animals. Most of this project is based on placenta tissue samples that we acquired through multiple national and international collaborations. However, to study some aspects of placenta development and function, we must work with genetically altered mice.

Why were they not suitable?

There are no feasible alternatives to studying the placenta outside its native (animal) context because we know too little about this system to approximate it using other ways. The development of the placenta is one of the most complex biological systems as it requires tight cooperation between two distinct individuals, the mother and the embryo. By studying the development of the placenta and its evolution across species, we aim to generate knowledge that will inform future studies and allow the development of *in vitro* and *in silico* models.

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 are aiming to breed 12 genetically altered strains of mice. We will breed these mice to maintain the strains in our animal facility, and we will set up specific matings within and between these strains to collect the placenta tissues (the pregnant females will be humanely killed). We estimated the total number of mice by multiplying the number of strains (12) by the number of mice that historically were required in projects with similar goals per year (80) times the 5 years (4800 mice).

We are aiming to maintain 10 species of Poeciliids. Of these, we estimate that we will need to administer hormones to promote matings to a maximum of 5 species. If we administer hormones to 40 females in each species per year, that will lead to 5 species x 40 females x 5 years, which is 1000.

We will also create a tissue biobank for the 10 species of Poeciliids. This means that in addition to collecting the maternal-fetal interface (i.e., the placenta in species that have this organ), we will also collect other tissues (e.g., brain tissues, kidneys, ovaries). Across the 10 species, we estimate that we will humanely kill and then harvest tissues for 500 individuals (50 per species) in order to cover both sexes and main developmental stages.

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

We established national and international collaborations to create a tissue biobank of hundreds of placentas from multiple species and developmental stages. The placentas were not collected specifically for this project. Through this step, we dramatically reduced the number of animals to be used in this project. We will keep using this strategy of collaborating with others to minimize animal use whenever possible. Additionally, to reduce the use of the Poeciliidae fish in future studies, we will collect tissues (in addition to the placenta) from humanely killed animals and create a tissue biobank.

We consulted with the staff at our animal facility, including veterinarians, to estimate the minimum number of animals needed to maintain genetically altered strains. We strive to use and maintain low numbers of animals by sharing lines and tissues from genetically altered mice with other labs and freezing embryos, tissues, and gametes whenever possible. Because our work focuses on prenatal development, when we humanely kill a female mouse (or administer a substance), we can obtain tissues from the entire litter of embryos. Each embryo is a data point. Together, this strategy will reduce the overall number of mice used.

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

Whenever possible, we will obtain tissues (placentas) through collaborations. We have done this successfully for animals from multiple species that are not genetically altered, and we will aim to do the

same for genetically altered mice strains. We will only breed them ourselves when this cannot be done through collaboration.

Breeding will be carefully controlled to avoid surplus animals, and when animals do have to be sacrificed, we will aim to collect as many tissues from as many animals as possible (after they are humanely killed). We will build tissue biobanks for the different strains that we and others can use in future work.

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 work with two groups of genetically modified mice. One group of mice have modifications that need to be activated using gene activating substances (e.g. hormones). Some of these mice will allow us to visualise under the microscope (e.g., using fluorescence) the cells where specific genes are active, which is key to understanding placenta development and function. These genetic alterations do not cause any harm to the animals before and after activation. Other mice will allow us to understand the consequences of altering gene function in restricted physiological contexts, for example, in specific cell types and/or developmental stages. We will use these conditional alterations so that the modifications will only be active in a restricted group of cells and/or stages to minimise the negative effects on the animals. To activate the genetic alterations, we will give the animals the gene activating substances using methods (like oral gavage) that only cause mild discomfort and for a very limited time.

The other group comprises mice carrying genetic modifications with the potential to impact the development or function of the placenta, such as the maternal immune response to pregnancy. We will breed these mice and examine the placentas after the animals have been humanely killed. We can breed these mice because although the genetic alterations they carry may impact the placenta, they have restricted effects allowing for successful pregnancies.

For the fish species, the use of hormones to encourage breeding are not expected to have any negative impact, including pain or suffering.

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

The mammalian placenta is an organ exclusive of mammals and can only be studied in mammalian species. However, because the placenta is only present before birth, most of the animals used will be at an embryonic or fetal stage of development. We will sacrifice the minimum number possible of pregnant females. Whenever possible, we will focus on embryos during the first two-thirds of gestation.

The Poeciliidae fish family is unique among animals because a placenta evolved independently nine times (for example, the mammalian placenta evolved only once, so all mammals share the same placenta). This means that uniquely in Poeciliids, we can compare multiple pairs of closely related species where one species has a placenta and the other does not. The Poeciliidae is a unique natural system that cannot be replicated with other species. We will study the fish before they are born, and we will sacrifice the minimum number possible of pregnant females.

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

The mice that we will breed will be monitored by highly trained animal technicians. There are ongoing efforts in our animal facility to test different bedding materials and shelters to identify those that maximise the animal's wellbeing. Different strains can have different preferences. We will apply the insights from these efforts to our mice to maximise their environmental enrichment. The mice that will be administered gene activating substances will undergo increased monitoring after the procedure. We will work together with fish specialists to maximise the environmental enrichment of our fish. If hormones need to be administered to encourage matings, we will precisely follow the instructions from the fish specialists and veterinarians and monitor these fish closely for the hours following the hormone administration.

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

We will follow ARRIVE 2.0, PREPARE and NC3Rs guidelines. The NC3Rs monthly newsletter will keep us abreast of new developments.

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

We will stay informed through the NC3Rs monthly newsletter to which we are subscribers and through in-person training provided by our institution. Advances in the 3Rs are also shared internally at our institution via AWERB and NIO.