Home Office

NON-TECHNICAL SUMMARY

Embryonic origins of heart disease

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

embryonic, cardiovascular, heart, circulation, development

Animal types	Life stages
Domestic fowl (Gallus gallus domesticus)	embryo, neonate, juvenile, 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?

To determine some of the mechanisms via which adverse conditions during prenatal development trigger embryonic origins of heart disease

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?

To diminish the burden of cardiovascular disease by understanding mechanisms better in order to identify plausible therapies for intervention

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

The outputs that we will see at the end of the project will be:

1. Data relating to cardiovascular function, cardiovascular morphology (structure) and underlying molecular pathways in chicken embryos incubated under control of challenged conditions (e.g. lower than normal oxygenation or hypoxia) with and without treatment (e.g. antioxidants);

2. Data relating to cardiovascular function, cardiovascular morphology and underlying molecular pathways in hatchlings raised from fertilised eggs incubated under control of challenged conditions (e.g. hypoxia) with and without treatment (e.g. antioxidants);

3. Data relating to cardiovascular function, cardiovascular morphology and underlying molecular pathways in juvenile/adult birds raised from fertilised eggs incubated under control of challenged conditions (e.g. hypoxia) with and without treatment (e.g. antioxidants);

4. Data relating to cardiovascular function, cardiovascular morphology and underlying molecular pathways in first and second generation birds raised from fertilised eggs incubated under control of challenged conditions (e.g. hypoxia) with and without treatment (e.g. antioxidants).

5. Publications as abstract, papers, reviews and book chapters of Points 1-4;

6. Dissemination of unsuccessful approaches or negative findings.

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

The work in this project licence is to be done in the chicken. The reason is because in contrast to all mammals (except primitive mammals that lay eggs or monotremes), the chicken permits some of the direct effects of adverse conditions during development on the embryo to be isolated, independent of effects on the mother and/or the placenta. Establishing direct effects on the developing heart and circulation means we can better identify mechanisms involved. This will improve intervention to guide future translation into human clinical practice.

In the short-term term (1-3 years), the data outputs of this project licence (e.g. changes in arterial blood pressure, heart rate, cardiac function) will inform us how adverse conditions during early life may increase the risk of cardiovascular dysfunction later on in the adult offspring. For instance, is the heart affected more than the vasculature? Which is worse and which may contribute more to triggering cardiovascular disease?

In the medium-term (3-5 years), the effects of the experiments in chickens will also inform our programmes of work and those of other researchers in other species, such as in the mouse, rat and the sheep. It will tell us which are the areas to focus on, and what similarities and differences there are between species. The idea is then to create a layered approach of understanding on the effects of adverse conditions during development on the cardiovascular system across the life course in different species. This will help to better translate our findings to the human clinical situation. For example, knowledge of common effects of an adverse condition in early life on the heart of the chicken, mouse and sheep may precipitate human benefit by designing a treatment with protection across species and thereby likely to be as efficient in humans.

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

The outputs of this work, including the dissemination of unsuccessful approaches or findings, will be maximised at several levels:

Scientific advancement and collaboration: In the longer term (>4 years), the data will benefit the design of therapies in higher vertebrate models of adverse pregnancy with a view to human translation and the design of clinical trials. This will be achieved via collaboration with experts in different fields within and outside the university. Therefore, the proposed work in this new project licence may hasten translation to relatively simple but novel human clinical interventions to not only treat the mother, but also her progeny. This will contribute to a reduction in the burden of developmental origins of heart disease, thereby having a positive clinical, economic and societal impact on health.

Dissemination of new knowledge. Other pathways to further increase impact will include contacting the funders and the University communications office to alert them of the potential influence for human health of the scientific findings. This will lead to press releases, which will be supported by radio and television interviews. In addition, the data will benefit the design of cures to protect the health of the unborn child. The proposed research is therefore likely to be of significant interest and benefit not only to researchers carrying out similar or related research in the field, but also to national and international researchers in other disciplines, such as biochemistry, pharmacology and nanotechnology, as well as cross-disciplinary teams in the pharmaceutical industry. To deliver translational benefit to the nation's health, wealth and culture we will adopt a number of strategies such as seeking patent protection for any new therapies or diagnostic biomarkers revealed by the research as well as actively engage with the commercial pharmaceutical and healthcare sectors to exploit our research at the earliest opportunity.

Species and numbers of animals expected to be used

- Domestic fowl (Gallus gallus domesticus): 10000

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 chicken is the ideal animal for the project as it permits isolation of the direct effects of adverse conditions during development on the cardiovascular system, independent of confounding effects of these challenges on the mother and the placenta (as in mammals). This will help us better isolate the direct effects of challenges to development, underlying mechanisms and therefore the improved design of possible cures.

The studies will focus on the chicken embryo, the hatchling and the juvenile/adult bird as the project is designed to determine the effects of adverse conditions on the cardiovascular system of the progeny across the life-course, from embryonic stages through to the adult offspring.

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

The project focusses on three life-stages of the life-course: the chicken embryo, the hatchling and the juvenile/adult bird.

In the chicken embryo studies, typically a fertilised egg will be exposed to control (e.g. normal air or normoxia) or challenged conditions (e.g. lower than normal oxygenation or hypoxia) during incubation and the chicken embryo studied in late incubation (e.g. at day 20 out of 21-day incubation period). The majority of the studies will be ex vivo (after death), i.e. in tissues isolated from animals followig Schedule 1 killing. A minority of studies will investigate the function of the heart and circulation under terminal or recoverable anaesthesia following surgery. Under terminal or recoverable anaesthesia, typically 1 experiment will be performed in any one embryo, lasting approximately 5 hours.

In studies in the hatchling, juvenile or adult bird, typically a fertilised egg will be exposed to control (e.g. normoxia) or challenged conditions (e.g. hypoxia) during incubation and the chicken embryo will be allowed to hatch. The majority of the studies in the hatchling (typically 1-2 weeks of age) or in the juvenile or adult bird (typically 12 weeks to 1 year of age) will be ex vivo, i.e. in tissues isolated from animals following Schedule 1 killing. A minority of studies will investigate the function of the heart and circulation under terminal or recoverable anaesthesia following surgery. Under terminal anaesthesia, typically 1 experiment will be performed in any one animal, lasting approximately 5 hours. Under recoverable anaesthesia, typically 3 experiments will be performed in any one animal, with 1 day rest in between. Each experiment will typically last 3 hours.

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

It is well established that incubation of fertilised chicken eggs in adverse developmental conditions, such as lower than normal oxygenation or exposure to glucocorticoids (steroids) can reduce embryonic growth and survival. Our own experience is that about 15% of eggs are not fertile (checked by candling) and about 10% of embryos incubated artificially under optimal and normoxic (normal air)

conditions die normally, usually stopping development within the first 5 days of incubation. Exposure to 14% hypoxia (lower than normal oxygenation) between day 1-19 of incubation reduces the survival further by ca. 40% and hatchability by 50%. Relative to embryos incubated under normoxic conditions, surviving embryos of hypoxic incubations show a ca. 25% reduction in body weight. Our experience with glucocorticoid therapy is that treatment of the chicken embryo with synthetic steroid hormones, such as dexamethasone or betamethasone reduces survival by 20% and it decreases fetal body weight by 20% by the end of the incubation period. Therefore, we expect similar adverse effects of incubations with periods of lower than normal oxygenation or treatment with glucocorticoids during the tenure of this project licence. We do not expect other adverse effects of experiments at the chicken embryo stage of the life-course.

We do not expect any adverse effects of topical administration of substances (e.g. antioxidants or vehicle) onto the chorioallantoic membrane (membrane that separates the embryo from the air cell within the egg) between day 1-20 of incubation, via a 1 mm hole drilled into the eggshell. The hole in the egg shell will be covered by a small piece of tape after substance administration. We have plenty of experience with this route of administration and we have not seen problems, such as infection, for instance in 5 years.

Conversely, between day 15-20 of incubation, administration for example of antioxidants, or vehicle via intravenous, intramuscular or intraperitoneal may cause bleeding. Intraperitoneal injection in birds also increases risk from flooding one of the air sacs. If air sacs are compromised and /or bleeding on any one day is estimated to be >10% of the estimated embryonic blood volume, the animal will be killed by a Schedule 1 method. Similarly, if blood loss could not be controlled, the animal will be killed by a Schedule 1 method. Embryonic blood volume can be estimated from comparison of the size of the embryo to our own historical library of sizes and weights of embryos at different stages of the incubation period.

Following hatching, growth of hatchlings of incubations under adverse conditions may also be slower compared to controls. For instance, our own experience is that the weight gain of hatchlings from hypoxic incubations at 6 months of age is reduced by an average of 1-2 g/day. Therefore, we expect similar adverse effects of incubations under sub-optimal oxygenation on post-hatching growth with or without embryonic and/or post-hatching treatment with glucocorticoids during the tenure of this project licence. Conversely, we expect hatchlings exposed to an obesogenic diet (a diet that makes you gain weight) to put more weight on than controls as they grow to adulthood.

Some protocols require treatment of hatchling/juvenile/adult birds with intramuscular injections (in the muscle). Intramuscular injections in juvenile/adult birds will be given in the pectoral muscle. This may cause transient pain.

Exposure of hatchling/juvenile/adult birds to an obesogenic diet may lead to fatty liver. We do not expect that this will affect the wellbeing or behaviour of the animal during the tenure of the protocol.

While under terminal anaesthesia, some protocols may require surgical implantation of probes or occluders, or the application of vessel occlusion. Some of these procedures may lead to unexpected bleeding. If more than 10% of the estimated blood volume of the animal is lost, then the animal will be killed by a Schedule 1 method.

A minority of experiments in the hatchling/juvenile/adult birds require surgery under recoverable anaesthesia. All surgical procedures will be carried according to the Home Office Minimum Standards for Aseptic Surgery. Through previous work, we have gained significant experience with work on birds following surgery. Pain killers will be administered as required, judging from the animal behaviour. In some animals, arterial catheters will be placed which allow for blood sampling. The health of the bird can then be monitored through measurements of blood gases. For example, we will be able to ask if oxygen levels in blood are normal?

Some protocols require study of the bird after removal of the nerve that is attached to the carotid body (denervation of the carotid bodies) or removal of the adrenal glands. Denervation of the carotid bodies does not produce any resultant harm. In fact, carotid body denervation is currently being trialled in human patients to treat hypertension. In contrast, removal of the adrenal glands can trigger adverse effects on blood volume and blood pressure over prolonged periods of time. Adrenal insufficiency in man and other animals is known as Addison's disease and these individuals through hormone insufficiency can develop low blood pressure over a period of months. To minimise this possibility, no animal will be studied and kept for longer than 1 month after surgical removal of the adrenal glands.

In the event of post-operative complications, such as a catheter being removed, birds will be killed unless such complications can be remedied promptly and successfully using no more than minor interventions. In the case of wound dehiscence (bursting open), uninfected wounds may be re-closed on one occasion.

We estimate that about 10% of birds will not be able to weight bear on one or both legs after one day's recovery from surgery. In this instance, the animal will be given supportive care via a sling and it will be monitored closely by taking morning and afternoon arterial blood samples. The animal's food intake and ability to pass faeces will also be closely monitored at least twice daily. Any birds that cannot weight bear on two legs for 72 hours despite continued pain killer care, appropriate food intake, defecation and blood glucose concentration will be killed by a Schedule 1 method or earlier if its condition deteriorates before this point.

For studies involving a scan of the heart in the conscious bird, the plucking of feathers under gentle restraint to expose any area will be minimised. Feathers will be plucked in the direction of their insertion into the skin to avoid skin rupture and discomfort.

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)?

Species: Chicken

Mild: 90%

Moderate: 10%

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

- Killed

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?

In some experiments, we must use whole animals or organs isolated from animals because function, for instance in the cardiovascular system, is regulated by complex networks, none of which have been reconstituted completely in computer models. The overall system is not well enough understood to make mathematical modelling useful.

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

There are no suitable non-animal alternatives to use in this project.

Why were they not suitable?

No non-animal alternatives are suitable to use in this project as none can model the effects of adverse conditions during embryonic development in programming an increased risk of cardiovascular disease in the adult progeny.

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 estimated the number of animals that we will use in this licence from experienced use of animals, as detailed in more than one retrospective review using this species.

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

The experimental design was created with the NC3R’s Experimental Design Assistant to ensure reproducibility using the least number of animals to satisfy statistical power for anyone measurable output.

To ensure the minimum number of animals is used in this project to address all objectives, we have considered the choice of species very carefully. The real ethical, biological and economic value of the avian relative to other experimental animal models, such as rats and mice, can be best appreciated by considering the lack of need for surrogate mothers or for control of the effects of litter variation or the effects on lactation, as is needed in mammals that give birth to litters and suckle their young. For example, in rodents, one litter irrespective of the number of pups in that litter, is considered as the experimental unit for many statistical comparisons. In addition, adverse pregnancy in mammals may affect the quality of the mother's milk. Therefore, additional control animals, such as surrogate mothers for newborn pups, are needed to understand these effects better. By using the bird as the species of choice to address the project objectives, this markedly reduces the number of animal groups, as there is no need to control for litters or maternal milk. Therefore, this significantly contributes to the 3Rs principle of reduction as enshrined in EU Directive 2010/63.

Where relevant, multiple experimental designs will be used, rather than the one-thing-at-a-time approach, to maximise the information obtained from the minimum resource. For most experiments, the study design will adopt methods of analysis previously published extensively by our group, which compares 4 groups: control and experimental groups with and without a treatment or intervention. For example, outcomes from normoxic (normal air) or hypoxic (lower than normal oxygenation) incubations with and without treatment with an antioxidant. Control groups treated and untreated are necessary, as the treatment may affect normal and complicated incubations differentially.

Sex differences are an important consideration in the risk of developing cardiovascular disease. Therefore, assuming a 1:1 ratio of males to females, the number of animals required per outcome variable will be doubled to be able to address sex differences. In such cases, statistical analysis able to compare three factors comparing treatment, intervention and sex will be adopted (e.g. a Generalised Mixed Linear Model; SPSS).

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

We will optimise the number of animals used in this project from 1) pilot data and 2) by using multiple data obtained from the same animal. For instance, we will obtain data from the living organism as well as from tissues isolated from them after death. In addition, we routinely share tissues generated from projects for collaborative studies by other investigators. About 20% of our publication output is derived from such collaborative studies.

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.

The work will be done in the chicken, which has several advantages. First, it permits isolation of the effect of adverse developmental conditions directly on the fetal heart and circulation independent of effects on the mother and the placenta. This helps better identify potential direct mechanisms and interventions on the developing individual. Secondly, most of the work will be done in the chicken embryo pre-hatching rather than in the post-hatching animal. Thirdly, the work is of greater relevance to humans than using rodents because the development of the heart and circulation is much more similar in humans and chickens, compared with humans and rodents. Fourthly, we can apply interventions in greater numbers of chicken embryos at any one time within one incubator, allowing for contemporaneous comparison and thereby minimising confounding effects of increased variability (e.g. experiments affected by seasonality, effects that sometimes cannot be avoided in longer living species).

A much larger component of the work can be achieved by investigating isolated organs and tissues. For example, after death, the function of the isolated heart and vessels can still be investigated, as well as experiments at the level of the cell and molecule. Comparatively smaller components of the work will involve studying whole living animals under terminal anaesthesia, or conscious animals which have been surgically prepared under general anaesthesia. In some cases, it is necessary to study conscious animals, as anaesthesia can impair normal cardiovascular function. Experiments will only be performed following appropriate post-surgical recovery. We will keep suffering to the minimum by using procedures with the least possible severity, and by subsequent monitoring with veterinary advice.

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

The development of the cardiovascular system in less sentient species, such as in worms or flies is very different than in humans. In addition, the regulatory mechanisms of cardiovascular function in such species is not well understood.

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

With experience from previous work, we have streamlined and refined surgical procedures that reduce bleeding, shorten anaesthetic exposure and improve port-surgical recovery. For example, we have slightly raised the bird's head during general anaesthesia to prevent inhalation of any crop reflux. We have also used a different surgical approach to refine procedures. For example, in some preparations, we will need to catheterise the femoral artery of the adult bird. We now do so by isolating the vessel on the outside of the leg muscles rather than through an inguinal approach. From past experience, we have found that this refined surgical approach markedly reduces the disruption of circulation, reduces bleeding and improves the post-surgical recovery of the bird.

In some birds, under recoverable anaesthesia and using strict aseptic conditions, catheters will be placed in one leg and a flow probe in the other. Following 5 days of postoperative recovery, experiments lasting 7-10 days will be performed in these chronically instrumented birds. Approximately 80% can stand following surgery, 15% are reluctant to stand but can stand and weight bear when prompted and 5% are unable to stand. A score sheet has been developed to assess mobility and behaviour to guide decisions, and it will continue to be used and improved as appropriate. The score sheet will be trialled for 6 months and it will be refined thereafter with the input from the Named Veterinary Surgeon.

Bedding in the animal holding areas has also been improved. Some forms of past bedding used in the facility created a lot of dust in the animal's environment. The new bedding material generated much less dust.

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

To ensure experiments are conducted in the most refined way, we follow the NC3Rs' ARRIVE guidelines, the LASA guidelines and the PREPARE guidelines.

For example, we will refer to the latest edition of the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery 2017, at the time of preparing this project licence application.

The Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines issued by NORECOPA (https://norecopa.no/prepare) covers all stages of quality assurance, from the management of an animal facility or population to the individual procedures which form part of a study.

We will refer to specific guidance or position papers from the Laboratory Animal Science Association, (LASA) https://www.lasa.co.uk/current_publications/. For example: Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T (2018) PREPARE: guidelines for planning animal research and testing. Lab Animal 52(2): 135-141. doi: 10.1177/0023677217724823.

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

We visit the NC3Rs website frequently and have subscribed to their newsletters.

Project licence holders are ultimately responsible for implementing the 3Rs within their work. Therefore, the project licence holder will have regular discussions with the Named Persons and animal technicians to review current approaches and whether there are any new 3Rs opportunities.

We will access our local NC3Rs Regional Programme Manager. We will maintain contact to obtain an informal route to 3Rs advice, developments, and best practice.

We will use of other resources, such as Norecopa https://norecopa.no/databases-guidelines. Regular consideration and reflection of the latest practical guidance from Laboratory Animal Science Association (LASA) will provide additional sources of new recommendations and advances in animal techniques.