Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 24

Projects with a primary purpose of: Basic Research – Urogenital and reproductive system

Project Titles and keywords

1. **Developing novel treatments for mitochondrial diseases**
    - Mitochondria, Mitochondrial quality control, Mitophagy, Drug screen

2. **Resource allocations and the cost of reproduction in animals**
    - Antioxidants, oxidative stress, reproduction

3. **Regulation of female fertility in health and disease**
    - Oocyte egg ovary fertility

4. **(Epi)genetic Programming of Growth and Metabolism**
    - Growth, Metabolism, Epigenetics, Transgenesis

5. **Molecular Regulation of Mammalian Development**
    - Genes, Oocyte, Development

6. **Maintenance of genome stability in stem cells**
    - DNA Repair, germ cells

7. **Neuroendocrine Control of Reproduction**
    - Pubertal timing, stress, neuroendocrinology, LH

8. **Fertility control in wildlife**
    - Wildlife, fertility control, immunocontraception

9. **Cystic kidney disease models and new treatments**
    - Cystic kidney, cilia, treatment

10. **Establishment of early pregnancy**
    - Pregnancy, embryo, superovulation, oestrus

11. **Pregnancy complications: targeted interventions**
    - Pregnancy, mouse, placenta, therapeutics

12. **Development and differentiation of germ cells in birds**
    - Poultry, biobank, fertility, reproduction, germ cell

13. **Regulatory RNA mechanisms in germ and stem cells**
    - Fertility, spermatogenesis, oogenesis & spermatogonial stem cells

14. **Identification of critical factor(s) required for optimised embryo development**
    - Identification, critical factor(s),optimised, embryo, development

15. **Assembly and function of the nuclear envelope**
    - Frog eggs, nuclear envelope

16. **Foetal growth in the pig**
    - Pig, foetus, placenta

17. **Tissue repair and scar formation in skin and ovary**
    - Wound, healing, scar, fibrosis, ovulation

18. **Observing and Studying How Amphibian Embryos Develop**
    - Amphibians, Embryo, Experiments, Training, Students

Project 1	**Developing novel treatments for mitochondrial diseases**	
Key Words (max. 5 words)	Mitochondria Mitochondrial quality control Mitophagy Drug screen	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Mitochondria are the powerhouse of the cell, generating energy for all life processes. Hence mal-functioning mitochondria can cause a broad range of severe diseases like neurodegenerative disorders or metabolic diseases. There are no curative treatments for these diseases. In the cells, mitochondria quality control is done via a recycling process called mitophagy coupled with generating new mitochondria to replace them. However when mitophagy fails it can lead to disease. This recycling process appears thus as an important process in mitochondrial disease. We wish to study mitochondrial quality control so that we can activate it to treat diseases due to a poor mitochondrial quality.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Nothing is known about the natural history of mitophagy from the very early stages of life to the fully developed organism. The first aim of this project is to unravel this and increase our knowledge on this important process. When this will be done we will aim at finding ways to either stimulate or inhibit mitophagy. We aim at doing this using drugs or by changing diet.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 2900 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There should be few if any adverse effects. Because we plan to cross strains of mice that have not been interbred before, we might have some unexpected effects. We have therefore described the limit on the breeding protocol as moderate. The animals will be terminated by a schedule 1 method at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have to work with mice because it is not possible to answer such questions in human or in tissue culture systems. Mitochondrial quality control in cells cannot imitate what happens in a whole animal at different ages.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals needed, we will run a parallel investigation using cell culture to identify useful drugs by screening a clinical drug library. We aim thus at repurposing drugs already available for clinical trials. Before starting any experimentation on mice we will use statistical methods to define the minimum number of mice to be used to produce reliable results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The main reason to use mice is that we want to study the mitophagy and mtDNA synthesis timeframe and modulation in the whole organism. The organism complexity cannot be reproduced by any *in vitro* system. The mouse is the animal of choice in studies on physiological pathways and genetic diseases because of its genetic and biochemical similarity with humans. Moreover to validate potential therapies, to be eventually translated to humans, it is essential to analyse their feasibility and safety *in vivo.* Cell cultures will however give important information on deciding which drug will be used in the mice, and experiments carried out on MEFs and human fibroblasts by us or other research groups will be the starting point for our work on mice. Mice are ideal because we have access to a strain that has red mitochondria and a green recycling system. Moreover a lot of different mitochondrial disease mouse models are also available so we will be able to

	test any potentially interesting compound. To prevent animals from suffering we will consult the NVS and use analgesics or anaesthetics during drug or BrdU injection if needed. Drug side effects will be closely monitored and treatment immediately stopped if the clinical signs are mild and transient (such as pilo-erection for <24 hours) and humane endpoint will be applied (schedule 1) whenever the animals are in distress because of severe effects (>20% weight loss, hunched posture, affected respiration pattern).

Project 2	**Resource allocations and the cost of reproduction in animals**	
Key Words (max. 5 words)	Antioxidants, oxidative stress, reproduction	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	High levels of investment in reproduction can reduce future reproductive capacity and survival (the so-called 'cost of reproduction'). However, the mechanisms underlying this association are poorly understood. A leading hypothesis is that metabolically-demanding processes such as reproduction can result in increased formation of unstable molecules called reactive oxygen species (ROS), which can cause serious damage to DNA, proteins and lipids and result in impaired cell and organ function ('oxidative damage'). But despite numerous studies on this topic, empirical evidence of such an oxidative cost of reproduction is equivocal. It has recently been hypothesised that previous studies may have failed to detect an oxidative cost of reproduction because mothers and fathers may pre-emptively reduce their own body levels of oxidative damage in readiness for breeding. Such 'oxidative shielding' could function to guard against levels of oxidative damage subsequently exceeding some critical threshold as a consequence of high levels of reproductive effort. Alternatively, 'oxidative shielding' could function to protect offspring from oxidative insults during particularly sensitive periods of development. This project aims to examine levels of oxidative damage and bodily defences against such	

	damage, in blood and organs of breeding compared with non-breeding birds sampled at the same timepoints. It will also provide the first detailed study of how the oxidative state of individual adults varies over the course of a breeding attempt. Finally, it will link variation in parental oxidative state to reproductive performance, and to the development and survival of offspring.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will lead to an improved understanding of the mechanisms by which reproduction is costly, and trades against future reproduction and lifespan. This will benefit other researchers, particularly those interested in evolutionary biology and animal ecophysiology, and those interested in more applied application of knowledge in the fields of medicine and veterinary science. Finally, since the costs of reproduction are of fundamental importance in the life histories of all organisms including humans, the results of the research are likely to be of interest to the general public.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a period of 5 years: Wild study of tits - up to 2000 adults and 3000 chicks Laboratory study of zebra finches - up to 700 adults and 600 chicks
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No adverse effects are expected. Relatively small blood samples will be collected using aseptic techniques. Individuals will be monitored at all times to detect any signs of stress or suffering. If individuals continue to suffer blood loss or extreme stress 20 minutes after blood sampling they will be euthanized using a Schedule 1 method. The procedure is of mild severity and no adverse effects are expected. Most birds will be released to the wild at the end of the study. A small number of individuals of laboratory-housed zebra finches will be killed using a Schedule 1 method to allow sampling of organs for markers of oxidative state.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	There is no alternative to using live, sentient animals, if we are to gain an understanding of the mechanistic basis of the cost of reproduction and how ecological factors shape such costs. Mathematical models can

use non-animal alternatives	provide a valuable theoretical framework for examining the cost of reproduction. However, for such models to lead to advance in understanding, it is essential that predictions are tested using empirical data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All of the proposed methods have been tried and tested many times before in numerous different species. There is therefore a very high level of certainty that the project will yield the data that are required to address its aims. Sample sizes have been decided based on power analyses to ensure that the minimum required numbers of animals are used. The research has been designed to make use of repeat sampling of the same individuals where possible – this yields greater statistical power, and therefore enables use of smaller sample sizes of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The projects will focus on small passerine species of birds which are numerous and widespread, and are known to be tolerant of observation and experimental manipulation during breeding. The only regulated procedure involved in the project is collection of blood samples for the measurement of oxidative state, and for molecular determination of sex. Blood samples will be relatively small, and any repeat sampling will be carried out with sufficient intervals to ensure that birds have fully recovered. The specific timing of blood sampling will ensure that individuals are not disturbed during the most sensitive periods of reproduction, e.g. during egg production. Welfare impacts will also be minimised by carrying out blood sampling quickly and efficiently. Bleeding will be controlled with gentle pressure, and once bleeding has completely stopped birds will be released from the hand, or placed back into the nest-box (always within 20 minutes of capture).

Project 3	**Regulation of female fertility in health and disease**	
Key Words (max. 5 words)	Oocyte egg ovary fertility	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	- to further our understanding of the role of the oocyte in fertility regulation - to understand the role of the oocyte in ovarian pathologies, such as premature ovarian failure - to further our understanding of how ovarian aging affects fertility	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	These studies will further our understanding of the mechanisms that regulate female fertility which is critical to developing fertility-promoting treatments for humans, agricultural breeding programs, breeding programs for endangered species and to identify targets to help control introduced species and potentially new contraceptives.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice have been chosen for these studies (approximately 14,000). The number of animals used for experiments will be around half of all animals bred since the only use we have for males is as breeders, and therefore, the majority are culled before weaning.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will	In the majority of cases, ovaries and in some instances blood and other tissues, will be collected from untreated and treated mice for analysis; this causes mild suffering as the animals will be either under terminal anaesthesia or have undergone	

happen to the animals at the end?	schedule 1. Ovarian tissue transplant experiments require surgery for the mouse host (severity limit moderate) but with appropriate care, mice are recover completely from the anaesthesia (as determined by movement and behaviour) approximately 40 minutes after surgery.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The regulation of ovarian follicle growth and development is complex and requires organization on multiple levels. To unravel the function of the oocyte requires the use of whole animals because the oocyte and follicle are mutually dependent and cell lines do not exist. Cell culture assays will be used wherever possible to characterise the function of molecules involved in oocyte-granulosa communication.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals required for each experiment will be carefully calculated and minimum numbers will be used to obtain statistical significance. All animals used will be used as efficiently collecting as much data as possible per individual to ensure that the use of each animal is maximised. Transgenic mice will be almost certainly be maintained as individual colonies unless they are readily available commercially and it is more efficient to purchase them. Each mouse line maintained will produce only the mice needed for the specific designated experiments and for maintaining the colony minimising the number of animals used. Bovine cells are also used since numerous ovaries can be obtained with no cost to animals from local slaughterhouses.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have been chosen for these studies because they are the species of lowest neurophysiological sensitivity that will allow us to investigate these scientific questions. It is also relatively straightforward to manipulate genes in mice. Substance administration will be via the least invasive technique with preference to mini-pumps over repeated injections.

Project 4	**(Epi)genetic Programming of Growth and Metabolism**	
Key Words (max. 5 words)	Growth, Metabolism, Epigenetics, Transgenesis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This projects addresses two main questions: why are so many babies born small for their gestational age? why are these babies niore prone to developing illnesses such as diabetes and cardiovascular disease? Fetal growth restriction (FGR) is a major public health issue because it affects 10-15% of all human pregnancies. The National Health System spends vast amounts of money when dealing with neonatal and post-natal complications of FGR pregnancies. To answer these questions we must have a full understanding of the mechanisms that control the genetically determined potential for growth. These include the genes that make babies grow and the interaction between fetal, maternal and placental signals that fine tune growth. In brief, the starting point of this project is to make mice that have growth impairment in utero (and/or after birth). We then study the placental, fetal and maternal adaptations (as well as postnatal) that occur in response to the growth impairment. This will lead to identification of new molecules that regulate growth. In addition, we can intervene to rescue the growth impairment in these models by a variety of means (e.g.	

	drugs, nutrition supplementation), thus paving the way for the identification of therapeutical agents to cure FGR, for example (currently there are no therapies for human FGR). We will also measure the metabolic/cardiovascular consequences of being born small or big, or being small or big during early infancy, by measuring for example how energy (glucose) is utilized and/or how blood pressure is controlled. We will also investigate issues related to the recent "explosion" in number of people affected by obesity and diabetes, which cannot only be explained by our genetic makeup. We know that it is the interaction between genes and the environment that ultimately causes diabetes and obesity. However, we know very little about how the environment provokes changes in the behaviour/activity of our genes. We think that the answer to this lies in natural "chemical" signals that are physically placed or removed from our genes (this is called epigenetics, literally meaning on "top of our genes") We will induce changes in these chemical signals (epigenetic marks) in mice that are exposed to sub-optimal nutrition, measure their metabolic and cardiovascular characteristics, and see how these epigenetic marks contribute to the phenotypes.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The major benefit of our research will be to provide new knowledge and understanding of growth diseases and associated developmental, physiological and metabolic complications. Other potential benefits relates to early diagnosis of growth and metabolic-related diseases and the design of new therapeutical agents to prevent and cure these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 8600 Embryo/fetuses, 17850 neonate/adults over a 5- year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In most cases our experiments cause only a small amount of distress or pain to the animals. However, some animals will have developmental defects associated with growth impairment (for example anemia, skin lesions, increased risk of neural tube defects and tumours) and others may become obese and diabetic. We have well established limits to how long we can keep animals to prevent them from becoming too ill. Therefore, once the phenotypes have been characterized the animals will be culled humanely as early as possible, thus reducing severity.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mice are required for this project because they are the oniy placental mammalian model system which is amenable to genetic manipulation in vivo. They also offer unique advantages when performing nutritional studies during pregnancy and metabolic studies during their life-course. The physiology of the mouse placenta, growth and metabolism shares important features with human, and can thus be used to model human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals we will do a lot of pilot work in cells growing in a dish, because the more we know about the gene we want to manipulate in the mouse the easier it is to plan our experiments involving mice and less mice will be used. We will also use and study materials taken out from mice as much as we can. We will use multi-integrated equipment that allows us to do several measurements in a single mouse. We will use the least number of mice to obtain conclusive results based on powerful new mathematical models that we are developing for our specific research questions.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the species of choice because specific genes can be manipulated (e.g. knocked-out) in either specific organs or the whole body (and importantly, the knockout mutation is passed on from generation to generation). This allow us to understand what these genes do during development of the animal — in our case, we are interested in genes that control growth and the roles they may play in the placenta (the organ that provides nutrients from the mother), for example. We have ways of following up the nutrients going from the mother to the fetus, and if a gene is very important in this process we should be able to identi1' it by studying a mouse that does not have that gene working properly in the placenta. We use analgesics and/or anaesthesia to minimize pain of some of our procedures and we use non-invasive techniques as much as we can (for example to measure blood flow from mother to fetus and from fetus to placenta, or to measure body fat composition).

<table>
<tr><td>Project 5</td><td>Molecular Regulation of Mammalian Development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genes, Oocyte, Development</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall objective of this project is to increase our understanding of the molecular processes regulating mammalian embryo development. Particularly we want to study the function of specific genes involved in this process.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The discovery that certain factors can ‘reprogramme’ mature cell types into stem cells has holds great potential for new biomedical applications, such as cell replacement, drug testing and disease research. Reprogramming allows us to turn any cell of the body into a stem cell. However the mechanisms involved in this technique are only just being identified and the success rate of the method remains very low. One way to improve our understanding of reprogramming is to study the natural programming mechanisms that begin after fertilisation in the mammalian embryo. In addition, basic scientific discoveries resulting from this programme of work will provide knowledge that would benefit couples undergoing assisted reproductive technologies such as in vitro fertilisation.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Mice Approx 2,650 over 5 years. The majority of these mice are adult mice; 2% are fetuses at later stages of</td></tr>
</table>

over what period of time?	gestation and neonates.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In order to provide eggs and early embryos, female mice will be given hormones to maximise the number of eggs and embryos produced. They are then killed by a humane method and the embryos collected, usually one or three days later. During the procedure these animals will only experience transient pain at the time of injection. Using molecular laboratory techniques we will identify and characterise the key factors involved in the regulation of gene expression during early embryo development in vitro – so thevast majority of the mice we will use are bred and killed for egg and early (up to day 4) embryo harvest and not subject to any invasive procedures. The ability to alter specific genes in the laboratory provides researchers with the opportunity to study the function of a particular gene. We will culture the eggs and embryos in specialist culture systems; assess their development and the effect of modified gene function. Very occasionally we may need to perform surgery on the mice, under general anaesthesia (for example to transplant some embryos into female mice or to perform a vasectomy on male mice). These are essential techniques (with moderate severity) but good surgical techniques, anaesthetic and pain relief will be used during and after surgery to minimise adverse effects. The mice are allowed to recover and will monitored closely post-operatively. Some mice will deliver at term and other pregnant females will humanely killed at specific time points in early gestation. At the end of the study all the mice will be humanly killed by an approved method. We will also breed genetically altered (reporter) mice (to obtain eggs and early embryos). These mice have been generated under other project licences. The effects of the genetic alterations (a fluorescent marker tagged to a protein of interest) are negligible, and the animals suffer no adverse side effects of this alteration. These mice will be superovulated and mated to obtain early embryos, experiencing no more than the same minor discomfort at the time of injection, as the control mice described above.
Application of the 3Rs	
1. **Replacement** State why you need to use animals and why you cannot	All proposed studies build on extensive data derived primarily from in vitro and biochemical studies carried out either by this group or by the international scientific

use non-animal alternatives	community. We will use mice mainly as egg/embryo donors with the remainder of the experimental work being carried out in the laboratory in vitro. We cannot obtain eggs or embryos without the use of live animals. We sometimes need to implant embryos into female mice to observe development in vivo because our current culture systems cannot support development beyond day 4 of development (i.e post-implantation development). In addition, reporter mice which have a fluorescent marker tagged to a particular protein of interest are an essential tool for molecular studies; allowing us to monitor and quantitate the reprogramming efficiency of our experiments in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental design is given priority with power analyses conducted prior to the research study to determine an appropriate sample size to achieve adequate statistical significance. Appropriate positive and negative control treatments are included where necessary. All procedures are carried out by highly-trained staff using well-established protocols to optimise experimental design. The number of egg and embryo donor animals used will be minimised by giving the animals hormones to increase the number of eggs they produce, this increase the number of eggs recovered by approximately fivefold. Careful statistical treatment of all data will be undertaken, gaining as much information as possible from each experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is selected as a model species for these studies for several reasons. Firstly, there is more information available about this species than any other in genetics, molecular biology and reproduction. Secondly, the short reproduction interval allows studies to be completed more quickly than in any other mammal. Finally, more consistent observations can be expected as inbred strains are maintained in a closely controlled environment. All mice undergoing surgery will receive pain relief (analgesia) and good post-operative care. Moreover all of our animals are housed under pathogen free, environmentally controlled conditions. Animals are routinely monitored for the presence of pathogens that could potentially lead to infections Our long term goal would be to replace the use of mice. However, currently there are no alternative in silico models or cell culture systems that can be used.

Project 6	**Maintenance of genome stability in stem cells**	
Key Words (max. 5 words)	DNA Repair, germ cells	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This PPL aims to uncover how genetic information is passed from one generation to the next without accumulating errors. At present we understand a great deal about how damage to DNA (the blueprint of life) is repaired in cells cultured in a test tube. However, we understand very little about the kinds of DNA damage that occur in an organism and how this damage is repaired. Here we will set out to answer basic questions about how a crucial set of cells deal with DNA damage in an organism. It is essential that we can pass on our DNA either in eggs or sperm, which arise from germ cells to the next generation free from errors. If we fail to do this then mistakes (or mutations) can be passed on and lead to disease in our offspring. We will determine what machinery is employed by these specialised cells to repair damage to the DNA. We will go on to ask if the requirements change as germ cells mature from when we are an embryo, to when we go through puberty and become adults. We will also ask what kind of mutations arise in the germ line and which machinery should have repaired those mutations.	
What are the potential benefits	Errors in the code of life underpin a very great many	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	human diseases – most famously cancer. However, very many other diseases occur because parents pass on mutations in their eggs or sperm. This problem increases the older the parent, with more mistakes being passed on. This project aims to uncover how those mistakes are usually repaired and how failure to repair those mistakes leads to problems for individuals in the next generation.
What species and approximate numbers of animals do you expect to use over what period of time?	We use mice and genetically modified mice in our research. We anticipate using approximately 44,225 mice over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will generate mice that lack the ability to repair damage to their DNA. These mice may have a predisposition to cancer. The best indicator of either of these diseases is weight loss – therefore mice with a genotype that predisposes to these diseases are identified early. We will carefully monitor those mice and cull them before they suffer any ill health when possible. We will also breed mice which lack the ability to repair damaged DNA. This may lead to developmental defects in their offspring. These pups will be carefully assessed as soon after birth as possible. Any pups that show defects that are likely to cause suffering will be culled as soon as possible. If a pup shows a defect of particular scientific importance guidance will be sought from the Home Office Inspector. We will perform bone marrow transplants and also transplantation of cells into the testes during this project. In order to achieve this we must prepare the recipients by conditioning – the same procedure is performed in humans before transplantation. As in the case of humans conditioning will involve giving mice chemotherapy (used in the treatment of human cancer) or exposing them to X-rays (used when treated certain cancers or autoimmune diseases). This may results in mice losing weight for up to 2-3 weeks before recovering. If mice do not recover then they will be culled before they suffer any additional ill health. Finally, in order for us to really assess the repair of specific kinds of damage to the DNA of the germ cells it will be necessary to expose mice to known DNA

	damaging agents. Following this process we anticipate that approx 70% of our mice may lose weight but they will be carefully monitored. Weight loss is a good indicator of the health of our animals. Animals that gradually lose weight will not be allowed to lose more than 20% of their body weight if other clinical signs are seen. If no other signs are seen animals may be monitored for a further 48hrs before they are killed only if their weight remains stable and no other signs develop.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is very difficult to study the production of sperm and eggs in any system other than a whole organism. This is because sperm and eggs cannot divide to produce more sperm and eggs since they only hold half of the amount of genetic information as all other cells. We also want to see how these cells begin life in an embryo. We will perform initial experiments by growing these cells in a test tube. However, this has limitations as the process of maturing germ cells in a test tube is distinctly different than what happens in an organism. The test tube germ cells do not undergo some processes that happen in a live animal. These processes can produce DNA damage, therefore we cannot always used this system to study the process of repair. Mammals are the most appropriate model because the way in which they make their germ cells is distinct from other vertebrates. We are particularly interested in the mammalian germ cells as they go through the same set of processes as human germ cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experimental design will ensure we use the minimal number of mice to achieve statistical significance. When embarking on work with many variables we shall carry out small pilot studies so that we can refine our experiments. Finally, we will cryopreserve our strains. This prevents us needing to keep all of our mouse strains alive just to perpetuate the strain.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	We will use mice due to their genetic tractability and similarity to human development and germ cell function. DNA repair deficient mice and pups born to them will

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	be monitored carefully by day daily inspection for clinical signs and also through weekly weighing when we suspect that they will develop ill health. Mice that are beginning to develop clinical signs will be culled and analysed before they reach the moderate severity limit whenever possible.

Project 7	**Neuroendocrine Control of Reproduction**	
Key Words (max. 5 words)	Pubertal timing, stress, neuroendocrinology, LH	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to determine the neurophysiological mechanisms regulating the timing of puberty and reproductive function in adulthood.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The consequences of altered pubertal development in both man and animals, whether early or delayed, is link to a plethora of reproductive, sexual, behavioural, mental or other health issues with lifelong social, health and economic implications. Further knowledge is needed on the basic mechanisms underlying puberty control, not only to improve social and health outcomes in modern human society with its greater prevalence of stress, but to increase the potential for applied aspects of puberty control in the farming sector to improve production, welfare and sustainability.	
What species and approximate numbers of animals do you expect to use over what period of time?	3,400 rats and 650 mice over 5 years	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The possible adverse effects of surgery are infection and pain. To eliminate these as far as possible, surgery will be carried out under aseptic conditions with the use of antibiotics to minimise the chance of infection. Animals will also receive preand post-operative analgesia and will be closely monitored by trained staff and additional analgesia given if required. At the end of the experiment the animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We shall use both in vitro techniques and animal procedures. However, animal work is essential as puberty and the ovarian cycles only occur in whole animals and therefore, cannot be studied in a cell line.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals used, we shall use the minimum number required according to statistical power calculations to give us a conclusive answer. We shall also use mathematical modelling to test our hypothesis, where possible, to prevent unnecessary animal experimentation.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are the lowest vertebrate group that will allow reliable electrophysiological studies and in which the reproduction control systems have been well characterised. Animal welfare is a top priority as poor health and wellbeing have an effect on reproductive biology. The animals will be assessed regularly by trained staff for possible health issues and these will be relieved, or if this is not possible they will be humanely killed.

<table>
<tr><td>Project 8</td><td>Fertility control in wildlife</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Wildlife, fertility control, immunocontraception</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Conflicts between wildlife and human interests may be resolved through the use of safe and effective fertility control agents by modifying one of the parameters (productivity) that influence population dynamic of overabundant species. The emergence of new technologies coupled with increased public interest in the area of fertility control for wildlife has led to a significant progress. Hence, we have now moved from proof of concept at the level of individuals to practical application. The current project seeks to build on these successes by evaluating population level consequences of using these tools and developing methods for oral delivery of fertility control agents. This research is expected to substantially increase the scope for practical application and realise the potential of fertility control for safe, effective and humane resolution of wildlife conflicts.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Fertility control is used to limit population growth in several species (including man, cats and dogs) and the general public tends to look favourably to this method. Fertility control could offer an effective, humane and environmentally benign means of wildlife management that would result in the reduction of wildlife populations considered</td></tr>
</table>

	overabundant and thereby conflicts in situations where the impact of wildlife on human and other interests, such as biodiversity, is considered unacceptable. This would also reduce the need to resort to lethal control and thus the adverse effects in terms of animal welfare associated with such techniques.
What species and approximate numbers of animals do you expect to use over what period of time?	Laboratory rat, laboratory rabbit, wild boar, feral goat, European badger and grey squirrel, with 300, 60, 20, 200, 135 and 80 used respectively in any one year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	It is possible that some fertility control agents might cause negative physiological effects. However, if such side effects seriously compromise the welfare of the animals concerned, then the study will be either suspended or modified. Possible side effects of drugs used to induce and maintain anaesthesia for restraint will be discussed with the Named Veterinary Surgeon. The number of times animals are anaesthetised will be kept to a minimum. Methods used to capture and monitor animals may cause injury. However, *t*hese risks are mitigated by the techniques being carried out to species-specific Standard Operating Procedures. These SOPs are informed by the literature, manufacturer's guidance and information collected from other users. The animals will be euthanized by appropriate Schedule 1 methods or will remain in the wild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The physiological effects of contraceptive agents can only be determined by whole body studies of living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Regular consultation with statisticians and with the AWERB will ensure that the minimum numbers of animals are used whilst still allowing statistically robust inferences to be made on the use of fertility control agents in wildlife management. Furthermore, data collected in this study are expected to offer the basis for population modelling of the effects of fertility control which will potentially reduce the scale of field studies required to demonstrate effectiveness. The best use will be made of the animals in the trials by employing principles of statistical design (replication, blocking)

	where appropriate.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The ultimate purpose of the project is to reduce the fertility of wild animals. Thus, whilst for some studies laboratory strains may be suitable as model species for proof of concept studies (e.g. laboratory rat and laboratory rabbit), some studies of particular target species inevitably means taking individuals from the wild e.g. badgers, grey squirrels or working with such species in the wild. All the species used in this study are present as one or more free-living populations in the UK and have negative impacts on human activities and/or conservation interests. As the effects of contraceptives can be species-specific it is essential to test these compounds on those species that are currently considered potentially suitable for management by fertility control methods. Every possible care will be taken to ensure that procedures are kept to the minimum to avoid suffering, distress or lasting harm. Wherever possible non-invasive techniques such as faecal sampling, hair sampling by hair traps, and video-surveillance will be used to assess the effectiveness and potential side effects of the contraceptives on the physiology, welfare and behaviour of the animals. Capture, handling and monitoring of wild animals will use the most refined methods available which will be tailored to the individual species concerned, based on the literature and experience of other users; and will be further refined by our experience.

Project 9	**Cystic kidney disease models and new treatments**	
Key Words (max. 5 words)	Cystic kidney, cilia, treatment	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We will assess the role and function of genes implicated in human cystic kidney disease and related conditions. The exact functional role of such genes and their encoded proteins remains poorly understood and there is a fundamental and clinical need to know more about these genes. In addition, this project will determine the response of cystic kidney disease to various proposed treatments.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work will generate novel insights into the disease processes underlying cystic kidney disease. A better understanding of cystic kidney disease may ultimately lead to novel therapies for human patients with these diseases. We aim to understand both the genetic effects that contribute to disease severity and the effect of certain potential disease modifying treatments using whole animals (mice) and assessing the progression of renal disease. This work builds on our understanding of cystic kidney disease from tissue culture models and allows us to move towards treating humans affected by ciliopathy disorders.	
What species and approximate numbers of animals do you expect to use over what period of time?	Whilst we seek to develop in vitro systems, currently whole animals are needed to faithfully model cystic kidney disease in a physiologically relevant manner and to fully investigate systemic effects of potential treatment regimes (for example, increasing fluid	

	uptake and administration of drugs which effect cell signalling pathways) requires whole animals. Mice are the most appropriate species due to the power of mouse genetics – enabling precise disease-causing mutations to be recapitulated and studied in vivo. Over the course of this 5 year project we expect to use 400 mice per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected adverse effects are related to the types of kidney disease of the mice, which will include progressive (but slow decline) in kidney function. This may result in increased water consumption, increased urination, mild-moderate dehydration and weight loss, or weight gain if there is fluid retention. Treatments will be designed to ameliorate kidney disease and are not expected to increase disease severity. For any procedure which might be associated with any discomfort, all animals will undergo general anaesthesia appropriate for the species. We expect a mild to moderate level of severity for all these experiments and at the end of the procedure animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to understand the disease process in cystic kidney disease we need to study model systems, since ethical concerns limit the studies that can be done directly in people. The mouse is a very useful model as it closely represents human disease and there is high conservation of genes between man and mouse. The mouse is very useful for developing novel treatments and provides a means for studying the effect in whole animals. These studies are required as a progression from ex vivo and cell culture studies which we have undertaken, and are required prior to studies involving patients
2. Reduction Explain how you will assure the use of minimum numbers of animals	Healthy heterozygous mice will be maintained at a minimal number in order to produce sufficient offspring for experiments. Some mice will be grown up in the animal house to replace adult mice that are no longer productive, allowing maintenance of the smallest number of healthy adults. Preliminary work in vitro will allow selection of the most potentially useful therapeutic agents.
3. Refinement	The mouse is a useful animal in which to explore kidney disease as it has a kidney with features

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	remarkably similar to the human kidney. We aim to understand the events that lead up to a kidney cyst developing by mimicking cystic kidney disease in the mouse and examining the consequences in detail. This level of information could not be obtained from patients. We wish to test treatments which change the activity of kidney cyst formation in mice. We will also test the ability of treatments to alleviate discomfort of disease and find out which treatments are most effective. Adverse effects will be avoided by optimising dose ranges of therapies in tissue culture models prior to animal experimentation and by using doses of treatments that are within physiological ranges. Animals will be carefully monitored on a daily basis for any adverse effects of treatments. We will also limit the severity of kidney disease by carefully monitoring the health of the animals and humanely killing those whose condition deteriorates and who are therefore in danger of developing more than moderate signs of pain or distress.

Project 10	**Establishment of early pregnancy**	
Key Words (max. 5 words)	Pregnancy, embryo, superovulation, oestrus	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand the physiological mechanisms associated with the establishment of pregnancy. Given that these are conserved across species, the results of this project will be of both clinical and veterinary value. Since the establishment of pregnancy is a rate limiting step associated with many experimental procedures (e.g. the breeding of specific experimental models of human disease), this project will also allow us to improve the methods used to achieve pregnancy and reduce both the numbers of animals used overall but also the severity of the procedures that they are subjected to.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are several potential benefits to this research: (i) For humans: this will highlight the mechanisms associated with the establishment of early pregnancy and highlight novel potential interventions for the management of infertility, recurrent miscarriage and preterm labour, as well as highlighting the inflammatory processes underlying a number of common disorders, such as autoimmune disease (e.g. rheumatoid arthritis) (ii) For animals: the methods developed and optimised as part of this project aim to streamline many laboratory protocols with a	

	view to replacing the use of vasectomised males and reducing the number of females overall in research programmes which use rodents as models (mice and rats). Moreover, it is expected that those that are still used will have refined, milder and less-invasive measures used for establishing pregnancy. This could benefit hundreds of thousands of animals worldwide per year as well as potentially having translational benefits for the farming industry in the longer term.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 1,920 Rat 360 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The adverse effects to these well-established procedures are minimal and the novel ones being developed will present a reduction in severity. The animals will be humanely euthanized at the end of each experiment. Pups generated from experimental females will be used in further experiments wherever possible in order to minimise the numbers of animals used overall.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mechanisms associated with the establishment of pregnancy are highly complex and systemic such that they cannot be studied in alternatives (e.g. cells/organs).
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be reduced in two ways: (i) by careful experimental planning with support from a statistician in order to minimise wastage and (ii) by improving existing experimental protocols used for achieving a pregnancy after embryo transfer.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals	The mouse is used as a model for other rodents as it is the least sentient species, yet remains a representative and useful model. Welfare costs will be reduced by using less invasive procedures (e.g. pessaries instead on injections) as well as ensuring that any animal used in an experimental procedure (none of which are more than moderate severity) is regularly monitored and receives pain control if appropriate. Animals will also be housed in social groups where they can exhibit normal behaviour.

Project 11	**Pregnancy complications: targeted interventions**	
Key Words (max. 5 words)	Pregnancy, mouse, placenta, therapeutics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Complications of pregnancy affect around I in 6 pregnancies in the UK and cause enormous social and financial burden. Some of the most common pregnancy complications include fetal growth restriction, preeclampsia and gestational diabetes associated with fetal overgrowth. Fetal growth restriction (FGR), relates to the inability of a baby to achieve its genetic growth potential. FGR significantly increases the risk of stillbirth and also leads to a greater risk of adulthood diseases such as heart disease. Preeclampsia (PE) is associated with increased maternal blood pressure and the presence of proteins in the urine, indicating sub-optimal kidney function. In addition, PE is associated with an increased risk of having an FGR baby making PE a high-risk pregnancy for mother and baby. Gestational diabetes mellitus (GDM) is characterised by excess glucose in the blood which arises during pregnancy and puts the baby at greater risk of being overgrown. An overgrown baby increases the risks of complications during delivery and, in addition, a baby that is overgrown at birth is at greater risk of obesity and diabetes in adulthood. Pre-term labour (PTL) results in early delivery of the baby and may result in FGR, also resulting in increased risk of childhood and adulthood morbidity, Thus, complications of pregnancy have implications that can last a lifetime. Despite these	

	devastating consequences, there are no treatments for FGR/PE other than early delivery of the baby, akin to PTL, which is itself associated with poor outcome. One of the reasons for this lack of therapeutics is that we do not fully understand the mechanisms underpinning these complications of pregnancy. It does appear that abnormal placental function is key to the onset of these complications but many of the exact mechanisms remain elusive. As such, it remains imperative that we continue to assess the changes in placental function that accompany these complications and to target therapeutics based on this evidence. As such, we have already demonstrated, in mouse models of FGR, that sildenafil citrate (Viagra) is one drug that may have therapeutic value in the treatment of FGR. We have shown that Viagra improves placental blood flow (which is often impaired in FGR) and increases fetal growth as a result. Following these data, a human clinical trial has been funded emphasising the potential for mouse models to provide a good pre-clinical testing ground. This project has 3 major objectives: 1 To identify whether signals derived from the fetus are important in the control of fetal growth and whether these signals are different/absent in cases of poor fetal growth 2. To identify new potential therapies for FGR/PE/fetal overgrowth/PTL by focussing on both dietary modifications and drugs already approved in the clinic for other diseases that share similarities to pregnancy complications (e.g. those designed to increase blood flow) 3. To target these therapies specifically to the placenta, both to minimise possible side effects of therapies and also to maximise the chances of success of these therapies. Targeting involves attaching ? protein 'tag' to these drugs which allows them to bind only to the placenta, maximising action of these drugs at the required site. This should minimise the risk of possible side-effects caused by drugs being delivered to multiple organs.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Following this project, there will be a greater understanding of the placental mechanisms that underpin FGR, PE, PTL and the fetal overgrowth associated with GDM. As part of this insight, we will have a greater idea as to whether signals from the fetus to the placenta are important in fetal growth and whether these signals are altered/absent in

	complications of pregnancy. This project also has the potential to identify further candidate therapeutics, in addition to Viagra. These candidate therapeutics may include dietary modifications such as beetroot juice, which contains ingredients (nitrate compounds) shown to improve blood flow, already deemed safe for use in pregnancy. In addition, we will have evidence as to whether drugs targeted to the placenta only, give additional benefit in terms of safety for mum and baby, and in terms of achieving greater therapeutic value compared with the same drugs given systemically, i.e. not targeted. Overall this work will increase the likelihood of human clinical trials of drugs to treat pregnancy complications.
What species and approximate numbers of animals do you expect to use over what period of time?	All experiments will be conducted in mice. We expect to use approximately 4000 mice across a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In our model of pre-term labour, we specifically induce PTL by targeting pathways known to be important in the onset of labour. This will allow us to test candidate therapies which we predict will delay this early onset of labour. The animal models used in this proposal have mild pregnancy phenotypes including high blood pressure (PE), diabetes (GDM) and reduced fetal growth (FGR). In terms of the administration of potential treatments, most of these will be administered via the diet (water or food) thus minimising adverse effects. For substances that are unable to be administered in this manner, this will be via an injection, either under the skin or into the abdomen (which may be of moderate severity). This will cause a mild and transient pain but injections may need to be repeated on several days. Our previous experience suggests that animals tolerate this well and do not show long-lasting effects. For all the drugs/therapies that we propose, we do not expect any adverse effects but animals will be monitored for signs of pain/distress should unexpected outcomes occur. For experiments when surgery will be required (e.g. insertion of blood pressure probes), the animals will be kept at a surgical plane of anaesthesia. Following this anaesthesia, animals will be brought around but pain levels controlled by the use of painkillers as required. Whilst we make every effort to use sterile techniques to minimise the risk of infection following surgery, this minimal risk remains. If this occurs, and the animal found to be in pain/distress, we will humanely

	euthanise the animal. Following all end procedures, animals will not be reused for any other procedure and will be euthanased humanely. associated with GDM. As part of this insight, we will have a greater idea as to whether signals from the fetus to the placenta are important in fetal growth and whether these signals are altered/absent in complications of pregnancy. This project also has the potential to identify further candidate therapeutics, in addition to Viagra. These candidate therapeutics may include dietary modifications such as beetroot juice, which contains ingredients (nitrate compounds) shown to improve blood flow, already deemed safe for use in pregnancy. In addition, we will have evidence as to whether drugs targeted to the placenta only, give additional benefit in terms of safety for mum and baby, and in terms of achieving greater therapeutic value compared with the same drugs given systemically, i.e. not targeted. Overall this work will increase the likelihood of human clinical trials of drugs to treat pregnancy complications.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	One of the reasons why so little progress has been made in developing drugs for pregnancy diseases is that clinical trials testing treatments in pregnant women are very difficult and ethically challenging. Thus, in order to assess the effectiveness of potential therapeutics, the use of animals is the only possible starting point. We always run experiments using human placenta in the laboratory alongside animal experiments as a first step in determining effectiveness in women but such experiments cannot inform us of any general beneficial or harmful effects to mother and fetus or their function when a blood supply is intact. Computer modelling of the pregnant woman is just not possible with our present state of knowledge.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will keep the number of animals to a minimum by making as many observations/measurements as possible on individual mice (also aided by the fact that each litter comprises multiple pups) and by removing as many tissues as appropriate for later analyses. This ensures that from one pregnant mouse, we can obtain multiple datasets. As we have several years experience of similar experiments on mice we can be confident of the

	minimum numbers we will need to achieve statistical significance. Experiments will be designed so that the primary statistical test will be to assess 2 variables, treatment (treated versus untreated) and genotype (genetically altered versus wild-type controls).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	As we require knowledge on placental function a mammalian species is essential for our work. The mouse has a uterus and placenta similar to that in women and also allows us to study genetically modified strains that have disease symptoms similar to those found in humans, Such accurate disease models are not available in any other species. We will minimise suffering by using anaesthetic for any potentially painful procedures and by careful monitoring of the animals to ensure they are not in discomfort. For recovery surgery procedures, analgesics will be used as necessary to minimise pain. Additionally, for administration of therapeutics, this will occur primarily via drinking water or in the food. Only if this is not possible, will injections or insertion of minipumps underneath the skin be employed. Whilst we do not expect any adverse reactions from our candidate therapeutics, the use of targeted treatments will further limit any off-target effects.

<table>
<tr><td>Project 12</td><td>Development and differentiation of germ cells in birds</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Poultry, biobank, fertility, reproduction, germ cell</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objective is to understand the genes that control how the earliest forms of reproductive cells - called “germ cells” – develop into sperm in males and eggs in females.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The project will bring an increased understanding of the fundamental aspects of human and animal reproduction that are common to all creatures with backbones. Understanding fertility in chickens is important for sustaining egg production and improving reproductive problems in chickens reared for meat consumption.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Chickens will be used, 7000 over a five year period. Many of these will be at the pre-hatched stage of development.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>Germ cells will be studied in the laboratory and also after they have been used to create fertilised chicken eggs, where they will develop like normal cells in an embryo. We expect to see a reduction in reproductive fertility, sexual differentiation and changes in sexual behaviour in some birds. The chickens that are produced will be maintained as special breeds or germ cell samples will be frozen so that the chicken breed can be brought back from frozen at a later date for further breeding or research.</td></tr>
</table>

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Germ cell development is a complex biological process in which the germ cell interacts with neighbouring support cells to form a viable gamete (sperm or egg). We have developed methods to culture germ cells in the laboratory so many experiments can be carried out in vitro. The normal development of germ cells, and the role played by specific genes, needs to be studied in the animal in order to study later developmental timepoints. This allows us to look at the interactions between germ cells and the supporting cells in the surrounding reproductive tissues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are developing improved cell culture medium which will increase of the efficiency of producing offspring from frozen reproductive cells. This will mean that that fewer animals will be used overall in producing offspring from frozen reproductive cells. When possible, the manipulated chick embryos will be evaluated before day 14.5 of incubation to establish if the novel conditions are likely to be an improvement over existing methods.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Using the chicken is a refinement because many developmental stages can be analysed in the laid egg before hatching. The genetically modified chickens produced in this study will usually have only one copy of the modified gene, which means they are less likely to have any adverse characteristics. They will only be bred to produce chickens with two copies of the modified gene in order to identify and analyse if and how the genetic change affects their physical characteristics and development. Animals will be closely monitored to determine if the there are any adverse effects of the genetic change and managed accordingly. For example they may be studied before the adverse effect develops.

Project 13	**Regulatory RNA mechanisms in germ and stem cells**	
Key Words (max. 5 words)	Fertility, spermatogenesis, oogenesis & spermatogonial stem cells.	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective here is to understand aspects of molecular biology that sustains germ cell development. The germ cells are the cell line that gives rise to the sperm and eggs cells that are responsible for the continuity of life and the genetic health for a given species. In this respect they merit special attention and are termed the 'immortal lineage' because it is the DNA from these cells that crosses that the generation barrier. Given the importance of this lineage in supporting the long-term health of species or society, it is imperative to understand both the biology of the germ cells themselves as well as the molecular processes that support them. In my laboratory we are interested in the spermatogonial stem cells, these are the cells that constantly supply the testis with cells that can differentiate to make mature sperm cells. We strive to identify cells in the testis with stem cell activity and how they support testicular function in normal or regenerative circumstances. We also investigate how aging affects germ cells. RNA and the processing of RNA is the molecular perspective that my laboratory focuses on. RNA is copied (transcribed) from the DNA and is an intermediate that is used as a template to instruct the construction (translate) the protein-based molecular	

	machines or structural components of the cell. However it is now appreciated the RNA itself is not just an inert or static intermediate but is a molecule that has great regulatory potential in cellular processes. We seek to understand how regulatory RNA and regulation of RNA through modification contributes to the spermatogonial stem cells and the differentiation of both male and female germ cells. Deregulation of these regulatory RNAs and RNA modification processes have been associated with many diseases both in and out of the germ cell lineage. Thus understanding of these molecular processes is important not only in understanding fertility disorders but also diseases that affect other organs.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The understanding of spermatogonial stem cell (SSC) biology bears the promise to deliver many potential benefits. The identification of the SSC populations *in vivo* in the mouse may enable the development of sorting strategies of human SSCs for cryopreservation from prepubescent cancer patients that will otherwise be rendered sterile by chemotherapy. This is a major quality of life challenge to this population of cancer survivors and its resolution is of practical clinical relevance. Furthermore the understanding of the circuitry of the enhanced self-renewal capacity of SSCs may reveal novel strategies for stem cell rejuvenation. The trend in the Western world is towards parenthood at older ages for both sexes. As with females, aging is also associated with reduced fertility potential in males. This is associated with decrease in sperm concentration, motility and morphological abnormalities. In addition it is now well accepted that the mutation rate seen as changes in DNA sequence in germ cells increases significantly with age. The SSC supports spermatogenesis throughout life and thus age related alterations in SSCs have direct impact on gamete quality. Therefore this research will deliver benefits to many aspects of male fertility, stem/germ cell aging and regenerative medicine. Here we propose to explore how RNA modifications changes affect functionality. Changes to the control of RNA function have been associated with human diseases or congenital disorders underscoring the importance of these modifications for basic human development and physiology. Therefore this aim delivers benefits that will advance knowledge on disease mechanisms as well as understanding of

	emerging facets of basic RNA molecular biology.
What species and approximate numbers of animals do you expect to use over what period of time?	Our studies use the mouse as a model genetic system given that the physiology, development and genome are closely related to that of humans. The mouse is also the most advanced genetic mammalian where gene function and contribution to disease and development can be understood. We project that 25,000 mice will be generated by breeding over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the mice will be used for breeding to assemble the desired combination of gene variants to explore the research objectives. Given the research here is concentrated on the germ line and the loss or defective germ cell development results in infertility that is not associated with physical pain or discomfort. A small fraction of animals will undergo surgery for spermatogonial stem cell transplantation with the use anaesthesia and analgesia. The procedures involved in this licence are predominantly breeding with some injections of substances to label cells or activate transgenes and are classified as mild. The surgery for stem cell transplant is categorised as moderate as it is a surgical procedure but in our experience it is well tolerated and animals recover rapidly. Animals will be humanely killed at the end of experimental procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, *in vitro* tissue culture spermatogenic differentiation systems do not exist. This necessitates of an animal model to study spermatogenesis. An *in vitro* spermatogonial stem cell (SSC) tissue culture system exists that is a powerful system for the analysis of this stem cell. My laboratory uses this model where possible to replace the use of mice and is employed to it maximum potential. However not all facets of stems cells can be studied *in vitro* and the culture conditions can alter their properties thus necessitating the use of animals to study SSC biology.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to reduce animal usage we will apply optimized experimental designs, statistical analyses and breeding strategies. Optimized experimental design will be employed to maximize the information from each animal and thus limit the subsequent use of additional animals. For example, should both DNA and RNA be required from a small population of ex-

	vivo isolated cells, then both nucleic acids will be sequentially isolated from the same sample rather than sacrificing additional animals that would required should the nucleic acids be isolated separately. Cell-based assays will be carefully optimized with wild type animals prior to application with genetically modified experimental animals thus reducing animal usage. We will use power calculations to reduce to a minimum the number of animals used in each experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the model of choice for the study of mammalian germ cell development, spermatogenesis and spermatogonial stem cells (SSCs) based on the fact that is genetically tractable system that shares much in common with human reproductive biology. The availability and use of genetically defined inbred strains enable the study of a gene mutation on a defined genetic background. The availability of precise genomic maps helps in the identification of homologous human genes. The majority of the assays or procedures employed in the laboratory has been refined over years and is now considered routine by trained staff. I will ensure that staff will be fully trained and supervised by myself. This protocol does not contain any severe procedures.

Project 14	**Identification of critical factor(s) required for optimised embryo development**	
Key Words (max. 5 words)	Identification, critical factor(s),optimised, embryo, development	
Expected duration of the project (yrs)	12 months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Poor fertility is a major problem in humans and domestic animals representing a significant economic loss for farmers and social discomfort to the couple. Currently 1 in 7 couples seek assisted reproduction technology (ART), with failure to implant a common reason for infertility. Similarly, in domestic animals, failure to produce offspring in a regular interval is a limitation to animal production and is one of the main issues for dairy cows. The establishment of pregnancy needs both the presence of a good quality embryo and a womb at the correct stage to receive it. To date, embryos produced in the laboratory develop better if transferred to the oviduct where the embryo will form after natural mating and spend early stages before transfer to the uterus for implantation. This shows that culture conditions are deficient and further scientific research is required to improve formulation of embryo culture media. Using funds from BBSRC, we have shown that a molecule called hyaluronan (HA) which is present in most mammalian tissues has an important role in embryo development. It is produced in different sizes in the reproductive system and by the embryos at later stages of development (blastocyst stage). We	

	have also shown the presence of Hyaluronidase (Hyal-2) (an enzyme which breaks HA into smaller fragments) in the oviduct. The small HA fragments can function as a survival factor and we have shown that they improve cow and sheep embryos quality and support their development to a later stage. Similar beneficial effects were observed when this enzyme was infused into the sheep oviduct. Enzymes with similar biological functions are delivered by spermatozoa to the reproductive organ during natural mating and are present at the fertilisation site in the oviduct. We believe that these enzymes or their end products have the potential of being used as supplements to commercial embryo culture media. We have designed experiments to test the effects of different Hyals on development and quality of sheep embryos produced in our laboratory from abattoir-derived ovaries. In addition, we will transfer the blastocysts to live recipient ewes to assess improvements in pregnancy rate after embryo transfer. In addition, we will be using human embryos donated to the research to investigate impact of Hyals and their possible use in human ART. This particular work does not involve animals and is not regulated under ASPA.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The unique feature of this project is the collaboration of an animal embryologist and a human assisted reproduction technologist. The main benefit of this project will be for improving efficiency of assisted reproduction technology both in human and animals including endangered animal species. The quality of embryos is a critical factor in determining pregnancy outcome after embryo transfer. We expect to find that hylauronidases improve embryo quality and result in higher pregnancy rate. This project will also investigate mechanism of action of hyaluronidases. Therefore, scientific community and clinician active both in animals and human will benefit from the data generated.
What species and approximate numbers of animals do you expect to use over what period of time?	60 sexually mature sheep
In the context of what you propose to do to the animals, what are the expected adverse	Surgical embryo transfer is a routine method in sheep breeding. The adverse effects are not specific to this procedure. Similar to any surgical procedure, there

effects and the likely/expected level of severity? What will happen to the animals at the end?	will be possibilities of fatality during anaesthesia or due to post-operative infection. Maximum care will be taken to work under absolute aseptic conditions. No fatalities occurred in animals which underwent a similar procedure carried out previously by the project leader. The animals will be killed at day 35 to confirm intact pregnancy outcome and to collect tissues for analysis, or they may be returned to the flock (subject to certain restrictions).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Current in vitro techniques are only able to predict developmental potential of embryos. In order to determine quality of embryos and their ability in establishment of pregnancy, it is essential to transfer the in vitro produced embryos to recipient animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals requested for the experiment was calculated using *in vitro* data and a statistical programme. In parallel to *in vivo* experiments, we will also carry out *in vitro* experiments using sheep uteri and ovaries collected from an abattoir.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For the experiments described in this project, sheep are the preferred experimental animal as they are one of the most similar animal models to human embryo development, so will provide more accurate results. Maximum care will be taken for the welfare and reducing suffering distress and pain of the animals. One cause of the animals suffering is using inappropriate method of handling during experimentation. Other cause of the animals suffering will be during administration of substances for injection of drug before or after surgery or during blood sampling. This will be minimised by using a needle gauge appropriate for sheep and the route of injection. Dose volumes will be kept to the minimum required to obtain results and within safe volumes. The surgery procedure will be performed under general anaesthesia. The animals will receive pre-emptive and post-operative analgesia to minimise discomfort and suffering.

Project 15	**Assembly and function of the nuclear envelope**	
Key Words (max. 5 words)	Frog eggs, nuclear envelope	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The cell's DNA, containing the genes, is separated from the rest of the cell by a membrane barrier, the nuclear envelope. This has an important role in organising the cell and controlling the genes. Degenerative diseases and ageing, as well as cancer, may be caused by mutations or disruptions in the proteins that are part of this barrier. We aim to discover how these proteins carry out their functions to give us clues about their roles in these diseases.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We will gain a better understanding of how cells are organised, how cells signal to their DNA and control gene expression, how cells are able to move (important for cancer research, for instance).	
What species and approximate numbers of animals do you expect to use over what period of time?	Xenopus laevis (African clawed frog) We have a colony of about 100 frogs which are each used 1-2 times a year to obtain eggs	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	This is a very mild procedure involving 2 subcutaneous (under the skin) injections of hormones. The hormones stimulate the frog to lay eggs in their natural way. Frogs are allowed to recover for a short time in a quiet, darkened area, before being returned to their normal	

end?	accommodation. Frogs are productive for many years but may be euthanized if they become sick or too old.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We only use the animals to obtain eggs which are laid via a natural process. The unfertilised eggs are then used to produce cell extracts, allowing us to do experiments that do not involve animals. The eggs provide large quantities of material in a very concentrated, stable and reliable form that has not proved possible to replicate from sources such as culture cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We only prepare extracts when they are needed and extracts are frozen and stored at -80'C, where they are stable for several years. As this is a mild procedure and the animals are ready to lay again after a few months, only a small number of animals are used in total.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	**Choice of species and methods:** Xenopus provide large quantities of eggs from each frog, the extracts are robust and produce reproducible results. These extracts were first developed over 30 years ago and are used by many groups worldwide for many different types of experiments studying many different aspects of cell biology, biochemistry and development. We have used them for about 20 years ourselves and published many peer reviewed papers on work using these extracts. I have also collaborated with several groups in the UK, Germany and the USA, who also use this system, tapping into their experiences of use and animal care. **Minimising welfare costs**: the animals undergo a mild procedure leading to a natural process (laying of eggs) and there is little, if any, suffering. To minimise stress while laying they are kept in individual darkened tanks. They are then allowed to recover in a holding tank for 24 hours before returning to their main housing tank. This allows them to finish laying eggs before returning to their tank so that their main tank is not contaminated with unfertilised eggs.

Project 16	**Foetal growth in the pig**	
Key Words (max. 5 words)	Pig, foetus, placenta	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of the project is to better understand how, and at what stage of pregnancy, some pig foetuses grow more slowly than their littermates. In particular, the project seeks to understand the relationships between placental blood supply and foetal size, and when such relationships are established.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A better understanding of the causes and timing of poor foetal growth could lead to new remedial strategies to reduce the incidence of low birth weight. These findings are also likely to be relevant to humans.	
What species and approximate numbers of animals do you expect to use over what period of time?	12 adult female pigs and 40 late gestation pig foetuses will initially be used on this project.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Ultrasound scanning will be performed on sedated or anaesthetised animals, or on animals that are familiar with the scanning environment, and so no adverse effects are expected. Foetal blood will be collected after the mother has been humanely killed and will be followed immediately by an overdose of anaesthetic	

	administered to the foetus. All animals will be humanely killed at the end of thework.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our studies describe relationships between the various components of pregnant pig foetuses, placentas and reproductive tract within the same uterus. This would not be possible to study using isolated organs, tissues or cells. We are currently freezing pig uterine tissue with a view to developing a pig uterine cell line, for future use in laboratory studies, when appropriate.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Following consultations with statisticians, we have conducted statistical power calculations, based on our extensive earlier work, and are confident that investigating 5 pregnant pigs at each stage of pregnancy will be likely to show statistically significant differences, where they exist. The use of a litter-bearing species (which can carry over 20 foetuses) enables statistical power to be increased by the use of within-litter comparisons (of foetal size, foetal sex and the interaction between foetal size and foetal sex). Furthermore, multiple tissues will be collected and measurements made from several feto-placental units from each pig.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The domestic pig has the most extreme naturally occurring foetal growth retardation of any mammal studied to date. This presents a problem for the pig industry, where weight at birth is the major determinant of subsequent survival and total weaning weight of the litter, and where piglets that are light at birth fail to thrive and present management problems throughout their lives. In addition, small pig foetuses can be characterised as both ‘small for gestation age’ (having proportional organ development), and ‘intra-uterine growth retarded’, which are typically characterised by brain sparing at the expense of other foetal organs. Both these types are identified in low birth weight human infants, and thus the pig is recognised as a valuable model to study human foetal growth. As a litter-bearing species, the pig provides an ideal model to compare foetuses of different sizes within the same uterus, free from the confounding effects of maternal genotype, husbandry or nutrition.

	During Doppler ultrasound scanning, suffering will be minimised by performing ultrasound measures either under sedation or anaesthesia immeciiately prior to euthanasia. Foetal heart blood will be collected rapidly and followed immediately by an overdose of anaesthetic. Foetuses will have limited capacity to suffer, as they will be anaesthetised by the overdose of anaesthetic given to the sow.

Project 17	**Tissue repair and scar formation in skin and ovary**	
Key Words (max. 5 words)	Wound, healing, scar, fibrosis, ovulation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This research project aims to understand how tissues repair themselves following damage. We are interested in how cuts to the skin heal, and how the ovary heals after ovulation. We strive to learn why some wounds fail to heal (e.g. in aged or diabetic patients), why scars form (and occasionally grow out of control), and why sites in our bodies subject to chronic damage and repair (e.g. the ovary) are more vulnerable to cancer formation.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project is of scientific significance since currently there is only a very incomplete description of tissue repair in the ovary. Also, there are many gaps in our understanding about skin wound-associated pathologies (including keloid scars and, non-healing ulcers). We have growing evidence that “master regulators” of gene expression may be mis-expressed in some of these clinical problems. This work will first define the expression of these proteins during normal repair, and will then investigate how they are mis-	

	regulated in disease. The expected benefits of this work, from a clinical standpoint, are numerous. Firstly, epithelial ovarian cancer, the 4th leading cause of cancer death in UK women (Cancer Research UK), and a current favoured hypothesis on ovarian cancer aetiology is incessant ovulation - that the successive bouts of tissue injury and repair, caused by the release of a fertile egg. Despite the long-standing nature of this hypothesis, we have yet to thoroughly characterise ovulatory wound repair; without this knowledge we cannot grasp how the process goes awry. Secondly, ovulation is associated with significant vascular damage and bleeding; consequently, patients with clotting defects are at risk of developing haemoperitoneum. An improved understanding of the haemostatic mechanisms at work during post-ovulatory wound repair may assist in their treatment. Finally, this work may shed light on ovulation problems, which in turn could impact on fertility. We hope to ultimately extrapolate our research to investigate these devastating ovarian pathologies in humans. The information that will be gained through our work on skin wound repair has the potential to benefit a wide variety of patients with wound-associated pathologies (e.g. non-healing ulcers, keloid scars, squamous cell carcinoma). Our work aims to inform the development of therapeutic strategies that will improve healing of acute and/or chronic wounds in humans, or will successfully treat pathological scars.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice to study both ovarian and skin wound healing, project with less than 500 being required over the 5 year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	In order to study ovulation in mice, we propose to inject them with hormones to trigger the ovarian cycle. This is a widely-used protocol that does not appear to cause any distress for the mice, as it requires only two injections and simply mimics the natural hormonal cycle of the mice (mild rating). Occasionally these

end?	animals may be treated with additional drugs (that are not expected to have adverse effects), or undergo a surgery so that we can manipulate the ovary (moderate severity, although animals are expected to recover quickly and unremarkably from the surgical procedure). As for the skin wounds, they are usually made to back skin, and typically 4mm in diameter (4 may be made on one mouse) or 1cm in length (2 may be made on one mouse) . Occasionally one 8mm wound will be made, or other sites of the body will be wounded to better reflect human diseases. This procedure is considered of moderate severity, but based on past experiences, we do not antipate any adverse effects (performed under anaesthesia with pain relief provided). All animals are humanely culled (Schedule 1) at the end of these experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexity of tissue repair cannot be accurately modelled in vitro or by using computer modelling because of the diverse cell populations and their intricate interactions. For example, recruitment of inflammatory cells from the circulation, or hormonal influence add layers of complexity that are impossible to fully recreate in a culture system. We are able to study certain aspects of wound repair with organ/tissue culture, and will do so whenever possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers are kept to a minimum by drawing on our own past experiences about inter-animal variability and important publications in the field regarding the number of animals required to answer our scientific questions with confidence. Numbers are further reduced as we are able to analyse numerous wound parameters with only one sample by making very thin tissue sections for histological analysis. The inclusion of intravital imaging to study post- ovulatory wound repair will also reduce the number of animals required. Finally, experiments are designed with a

	staged approach so that early findings may guide and inform us as to whether or not follow-up experiments are worthwhile and justified.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse has been chosen since it is mammalian (closely modelling the human situation), and offers opportunities to directly test the function of specific genes in the repair process with transgenic animals. In order for direct comparisons to be made with our previous studies of repair in mice, this on-going research would best be performed in the same models. In order to minimise welfare costs to the animals, all surgeries (skin wounding procedures) are performed under appropriate anaesthesia and analgesia. Adverse effects on overall mouse health are not anticipated, but nonetheless, all animals undergoing procedures will be monitored very closely, and if they exhibit physical indicators of distress or compromised well being, the veterinarian will be consulted and/or the mice will be culled according to defined humane endpoints.

<table>
<tr><td>Project 18</td><td colspan="3">Observing and Studying How Amphibian Embryos Develop</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Amphibians, Embryo, Experiments, Training, Students</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">This Project aims to teach University students important general principles of embryology and developmental biology of backboned animals by observing and studying in spawn of these amphibians the formation of properly formed embryos from fertilisation to moving tadpoles.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Much of the novel developments in modern medicine relate to developmental biology processes, such as embryology, stem cell biology and human diseases such as cancer. Good training of the next generation of developmental biologists will therefore benefit future biomedical scientific research into embryological birth defects, regenerative medical therapies and even fundamental cancer biology.</td></tr>
<tr><td>What species and</td><td colspan="3">Two different species of African anuran amphibians</td></tr>
</table>

approximate numbers of animals do you expect to use over what period of time?	called *Xenopus laevis* and *Xenopus tropicalis* will be used to produce spawn in which to observe and study embryos. To do this, we will need to use less than 50 females and 10 males per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will administer fertility hormone treatment to adult Xenopus to encourage egg and sperm production, respectively, followed if necessary by in vitro fertilisation to produce developing embryos for observation and experimental study. The students will only use stages of embryos that are not subject to ASPA legislation. The hormone treatment on adult Xenopus is subject to ASPA legislation and involves a subcutaneous injection, which is a mild procedure. No surgery or other invasive procedure will ever be required.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Providing the important practical training in general principles of embryology and developmental biology of backboned animals on amphibians represents replacement of more invasive and potentially harmful procedures on animals that are more related to humans but who like us, bear their embryos internally. We have also carefully considered Chick eggs, but found constraints with keeping embryos alive in opened eggs and with ensuring early stages would be available for observation. Cultured stem cell experiments can only replace some whole embryo experiments in research and are technically much too difficult for teaching purposes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are reducing the overall use of Xenopus animals at our University by coordinating collaborations and arranging sharing of produced eggs and sperm among different research and teaching requirements. We also accustom our Xenopus animals carefully to the hormone treatment so that many of them can undergo the treatment several times, thereby reducing the overall number of animals needed in total.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Choosing amphibian species for this training purpose was a carefully considered compromise to optimise the balance between academic benefit and animal welfare costs. Environmental enrichment of the animals' accommodation contributes to their increased welfare, fertility and quality of the produced eggs and sperm.