Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 25

Projects with a primary purpose of Basic Research - Urogenital/Reproductive System

Project Title and keywords

1. **Anti-mullerian hormone immunoassay development**
    - Monoclonal Immunoassays Anti-Mullerian Hormone

2. **Mammary gland development, stem cells and breast cancer**
    - Cancer, mammary, stem cells, genetics

3. **Production of fertilised oocytes**
    - Xenopus, oocyctes, multisensory, locomotion, CNS

4. **Neuroendocrine Control of Reproduction**
    - Reproduction, Kisspeptin, GnRH, Hypothalamus, Pituitary

5. **Use of a natural biomaterial for hypospadias repair**
    - Biomaterial, urinary tract, hypospadias, urethra

6. **Germline genetic inheritance and reproductive health**
    - Fertility; Ageing; Chromosomes; Mitochondria; Cancer

7. **Modelling complications of early pregnancy**
    - Pregnancy, Miscarriage, Ectopic, Maternal Health

8. **Cytoplasmic regulation of mRNAs**
    - Gene regulation, mRNA translation, post- transcriptional control, RNA-binding protein, translational activators

9. **Regulation of ion transporters expressed in the kidney**
    - High blood pressure, Salt intake, Salt excretion, metabolic syndrome

10. **Nerve growth and regeneration**
    - Stimulating nerve growth and guidance

11. **Mechanisms of meiosis and causes of aneuploidy**
    - Meiosis, oocyte, aneuploidy

12. **Renal inflammation, fibrosis and repair**
    - inflammation, fibrosis, repair

13. **Investigation of gonadal development**
    - Fertility, ovary, testis, embryo

14. **Fertility control in wildlife**

- Wildlife, fertility control, immunocontraception

15. Receptor functions in development and infection

- Receptors, fertility, pathogens, vaccines

16. Oocyte epigenetics: mechanisms and effect of diet

- epigenetics, methylation, oocytes, embryos, diet

17. Mechanism and functional role of calcium signals

- Calcium; cell signalling; fertilization; transgenics

18. Papillomavirus Life-Cycle Regulation

- Papillomavirus, Epithelium, Tropism, Warts, Regression

<table>
<tr><td>Project 1</td><td>Anti-mullerian hormone immunoassay development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Monoclonal Immunoassays Anti-Mullerian Hormone</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>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The project relates to the measurement of anti-mullerian hormone (AMH) an ovarian product which has numerous clinical applications in animal and human fertility work and monitoring some forms of ovarian cancer. The blood levels relate to the number of eggs the female individual has left and in the case of women allows prediction of the time to menopause. In previous work the applicant has developed antibodies used in the current market leading manual test for AMH sold by Beckman Coulter and upcoming automated assays by Beckman and Roche. These tests measure total AMH. In the proposed new

Project one goal is to make new antibodies which allow the measurement of the concentration of only the bioactive form of AMH. This is likely to provide additional information and one of the goals of the work is to provide tools both for further research and use in clinical and diagnostic tests. A second goal is to optimise AMH tests for a variety of animal</td></tr>
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	species where AMH has been shown to be useful in breeding studies of both commercial and endangered species. It can identify the females with the most eggs remaining.
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)?	New immunoassay tools could improve the clinical diagnostic value of AMH measurement in patient samples. In particular it is possible that abnormalities in the way AMH is processed to the bioactive form may underlie some cases of infertility or premature ovarian ageing and influence the age at which different women reach menopause. This is because AMH is involved in the control of how rapidly female animals and humans use up their finite egg supply. The existing AMH assays are already in routine use for tailoring FSH dose in routine IVF to the number of eggs the woman has, in predicting the time to menopause and in predicting the possible loss of fertility after treatment for cancer. AMH assays are already in use in animals to prevent dogs and cats undergoing unnecessary surgery by confirming their spay status and in predicting which animals have the highest ovarian reserve and likely to give the most eggs in farm animal breeding programs. It is believed that differing circulating levels of AMH in blood in the male and female fetus affect brain development explaining the sex bias in autism. It would thus be of interest to measure both total and bioactive AMH in newborns to see if there is any correlation later in life with any disorders of the brain e.g. autism.
What species and approximate numbers of animals do you expect to use over what period of time?	Maximum 200 mice in duration of the 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	The animals will be injected by several routes on a number of occasions and have small blood samples taken for testing. The procedures are rated as of moderate severity and the possibility of severe side effects is very low. Animals will be killed by a Schedule 1 procedure at the end of the procedure.

end?	
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For projects of the type described use of conventionally made mouse monoclonals is the most scientifically appropriate method of obtaining the necessary antibodies and the most likely to succeed. The applicant has proven their ability to generate very useful clinical products employing these same methods in earlier work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that the mice which we immunise are generating good immune responses and receive good final boosting to ensure adequate number of antigen specific lymphocytes in the spleen. This will prevent the need to repeat work as success is more likely at the first attempt. We will stage the immunisations using the method thought most likely to work first.
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 mice are to be used to make monoclonal antibodies and are the appropriate species for this work. Ordinary inbred mice will be used for most studies with occasional use of a genetically modified mouse strain lacking AMH where it is expected to give a wider range of antibodies. The number, route, volume and composition of injections will be chosen with animal welfare in mind. Anaesthesia will be used where it is necessary to minimise the stress in procedures and where the application of anaesthesia is less stressful than the injections.

Project 2	**Mammary gland development, stem cells and breast cancer**	
Key Words (max. 5 words)	Cancer, mammary, stem cells, genetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	The overall aim of this work is to further understanding of the mechanisms that control cell death and lineage commitment in mammary gland and in breast tumourigenesis and to use this new knowledge to develop better treatments for breast cancer. We aim to address five specific research questions. Firstly, what is the identity of, and hierarchical relationship between, subgroups of stem and progenitor cells? What are the mechanisms of cell death during involution of normal breast tissue? What is the cell of origin of breast cancers and is it the cell of origin or the initiating oncogene that determines the type of breast tumour that arises? What is the role of the tumour microenvironment in tumor progression and in particular, what is the role of immune cells and the tumour stroma? Finally, can we develop a useful 3D *in vitro* cell culture model of the breast that can be used for drug screening and basic biology studies and thereby reduce the number of animals used? The	

	answers to these questions will further knowledge on essential developmental processes in mammary gland namely, lineage commitment in stem and progenitor cells and the mechanisms of cell death during involution. In addition, we will gain vital information on breast cancer initiation and progression and discover new methodologies for the treatment of breast cancer especially the more aggressive apoptosis-resistant cancers that are currently difficult to treat.
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)?	Outcomes from this project will not only further knowledge of normal mammary gland development and breast cancer initiation and progression but will provide potential novel therapeutic tools for the treatment of breast cancer. This will be relevant to both human and animal breast cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and estimate numbers to be about 30,000 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?	Most animals that will be used will not undergo any procedure other than breeding. Many of these mice will be genetically altered but few will have any harmful mutations. The expected level of severity is mild. The majority of animals will experience, at most, a small number of injections, For a small number of experiments, mammary cells or tissue will be transplanted to mammary glands surgically divested of endogenous epithelium. For the breast tumour studies, mice will develop breast tumours and some may have metastatic disease particularly in the lungs. The severity limit will be moderate. However, the breast tumours will cause little pain and suffering unless they ulcerate in which case the mice will be humanely killed. Extensive lung metastasis will cause difficulty breathing and such animals will be killed. At the end of the studies, the mice will be killed by a schedule 1 method or killed by perfusion fixation when tissues are required for histological examination.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are interested in understanding the basic biology of the mammary gland and how breast cancer arises. The breast in a complex tissue that is made up of many different cell types including the milk-producing cells, fat cells and immune cells. The breast undergoes phases of rapid growth during pregnancy and equally rapid cell death during weaning. It is not possible to mimic this in a culture dish. However, we are currently developing a 3-dimensional culture model of the mammary gland that can be used to replace some of the animals that would be otherwise be required in this project. Our ultimate aim is to replace animals for the screening of breast cancer drugs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This project will require complex genetic crosses. We will design the mating protocols to maximise the number of mice with the correct genotype and will keep the number of mice as low as possible in the initial matings until the desired genetic combinations are achieved. We will use mice of the incorrect genotype as controls. For the tumour studies, we will use power calculations to determine cohort sizes based on preliminary data indicating the difference in size of tumour that might be expected.
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.	We will use the mouse for all these studies. Our work requires deletion of specific genes and the mouse is the only model that we can use to carry out genetic studies in the mammary gland. Inducible conditional gene targeting will be used to delete the gene under study only in the mammary gland wherever possible. This will provide better experimental data but will also reduce any adverse effects from deleting the gene in other tissues and thus provides a refinement. Most of the animals will be used in the breeding protocol that is in the mild category and animal suffering will be minimal. In the cancer studies, which will have a moderate severity limit, the size of tumours will rarely be larger than 1.2cm at the widest point and primary tumours may be surgically removed in the metastasis studies to reduce suffering.

<table>
<tr><td>Project 3</td><td>Production of fertilised oocytes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Xenopus, oocyctes, multisensory, locomotion, CNS</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></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></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The purpose of the PPL is to allow the breeding of Xenopus toads in order to generate tadpoles for use in non-regulated studies.

The overall objective of the research is to use a simple vertebrate animal, the young tadpole of the amphibian Xenopus laevis, as a model system in which to try to understand how nervous systems control movement. The specific aim is to discover how the tadpole detects different sensory stimuli and combines this information when making simple decisions about whether, and if so how, to initiate an appropriate movement.

Questions still remain about the detailed operation of the central nervous system (brain and spinal cord) in controlling movement, and how this ability emerges during early development. Because of the relative simplicity of the amphibian tadpole nervous system, and because these animals can be available at all times of year for experiments, their nervous systems are now among the best</td></tr>
</table>

	understood within the vertebrates and are providing basic information which has proved difficult to reveal in other vertebrates particularly mammals. This knowledge is needed to help provide insights into the normal functioning of more complex animals, and therefore into treating impairments of these functions in more complex animals including man.
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 results of experiments on the embryos, which are not themselves regulated as the animals are not sentient, provide detailed basic data on the operation of the early developing vertebrate nervous system which advances our knowledge of the normal functioning of such nervous systems. Specifically, the aim of the experiments is to understand how information from different sensory systems is used to control locomotion, and particularly the decision to initiate bouts of locomotion. These data will continue to inform studies using more complex animals and in turn will benefit those other animals and humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Species: *Xenopus laevis* Expected use: Approximately 100 adult animals over the five years of the proposed project. (Unregulated experiments on embryos/hatchling tadpoles will involve approximately 1000 animals 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?	In the context of what we propose to do, we do not expect any adverse effects on the adult toads used for regulated procedures. The expected level of severity is mild. As long as they remain healthy and continue to produce fertilised oocytes, animals will be re-used at intervals of no less than 3 months. Otherwise, animals will be killed by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	We aim to understand the operation and development of vertebrate nervous systems. We therefore study a simple vertebrate animal: the

use non-animal alternatives	living *Xenopus laevis* embryo. It is essential to use live embryos to study how their nervous systems function, rather than simply how they are constructed, and essential for live adults to be used to produce these live offspring. Hormones must be used to induce breeding as this species does not breed naturally in the laboratory. While computer models are a valuable tool, there is insufficient experimental data to make these a sufficiently powerful alternative to replace animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure the use of a minimum number of adult *Xenopus laevis* for production of fertilised oocytes, we will continue to breed from adults that have been shown to have good fertility and re-use them at minimum intervals of three months. We will raise embryos from each batch of fertilised oocytes at a range of temperatures to maximise the number of days (typically 4) over which they can be used for non-regulated experiments. This will minimise the number of procedures needed and, in turn, the total number of adult breeding animals needed.
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 choice of animal model (hatchling tadpoles of *Xenopus laevis* prior to the stage of free-feeding) was based on their common ancestry with mammals including humans but the relative simplicity of their nervous system and their experimental tractability. This combination maximises the likelihood of being able to meet the objective of understanding the mechanisms by which sensory signals are integrated in the nervous system to initiate and control movement. Welfare costs will be minimised by using only a mild procedure to control natural breeding of adults. As a possible further refinement, we will try out a newly published method in which breeding is controlled by adding steroid hormones to the water rather than by using injections of HCG (see protocol 2).

<table>
<tr><td>Project 4</td><td>Neuroendocrine Control of Reproduction</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Reproduction, Kisspeptin, GnRH, Hypothalamus, Pituitary</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</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>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Problems with fertility affect 20-30% of couples in Western Europe at least once in their lifetime. Even after seeking medical treatment, 9% of couples remain infertile. In industrialized countries, problems with fertility are often exacerbated by delaying child bearing, as well as poor diet and lifestyle choices. Normally, diverse signals reach the brain to report the achievement of developmental landmarks, to reflect food intake, and to monitor light cycles, hormone cycles and stress, all of which influence reproductive function. These signals coalesce upon a network of neurons to influence the reproductive system through the release of a brain hormone called gonadotropin-releasing hormone (GnRH). Disruption of these signals leads to irregular GnRH hormone output and decreased function of reproductive system. GnRH neurons require activating signals to initiate puberty and regulate the reproductive cascade, but lack the ability to directly sense many signals. Recently, another brain hormone called kisspeptin has been found to directly control GnRH release. However, multiple populations of kisspeptin neurons appear to play distinct roles in co-ordinating GnRH neuron functions. Despite the discovery of kisspeptin as a regulator of GnRH neurons, and therefore reproduction, very little is known about the biology of kisspeptin neurons, or the mechanisms that regulate their function. Appropriate GnRH receptor function is essential for the success of reproductive treatments that target the brain. However, the human GnRH receptor has</td></tr>
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	unique structural features that are not present in common laboratory animals. Limited physiological studies in humans lack mechanistic information regarding GnRH receptor functions in system with an intact reproductive cascade. A possible alternative is to use humanized animal models, in which the mouse gene, in this case the gene for the mouse GnRH receptor, is replaced with the human counterpart. This can provide valuable information in a living organism about normal receptor physiology, as well as about complications arising in pathophysiology. Accumulating evidence suggests that defective trafficking of the receptor, rather than other functional deficiencies, largely cause reproductive problems arising from human GnRH receptor dysfunction. The objectives of this project are to characterize signals that control kisspeptin neuron activity, and to determine how GnRH receptor physiology influences reproduction. More specifically, we will functionally characterize three kisspeptin cell populations that exist in the mammalian brain. To achieve this, we will use transgenic rodents to (a) establish global gene expression profiles for each kisspeptin neuronal population in different sex steroid environments to identify key factors that are important in their function, (b) create anatomical maps of the cellular connections that regulate each neuronal population, (c) identify the signals relayed through these connections to regulate kisspeptin neurons, and (d) determine the role of each kisspeptin cell population in controlling reproductive physiology and associated behaviours. Our second aim is to evaluate new strategies for treating GnRH receptor dysfunction that leads to infertility in patients. To achieve this, we will use humanized rodents to (a) characterize the effects of GnRH and gonadal hormones (e.g. estrogen, testosterone) in the regulation of human GnRH receptor and its function, and (b) test new therapeutic compounds in treating reproductive dysfunction caused by mutated forms of the human GnRH receptor.
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 hierarchy of signals and molecular mechanisms utilized by brain circuits controlling reproduction must be understood to harness the therapeutic potential of directing pharmacological interventions aimed at alleviating central causes of reproductive dysfunction and infertility. Targeting treatment upstream of the GnRH neuron to restore appropriate GnRH function may be used in

	conditions of decreased luteinizing hormone (LH) production such as hypothalamic amenorrhea and delayed puberty, or in conditions of high LH pulse amplitude and frequency such as precocious puberty and polycystic ovarian syndrome. In addition, repurposing cell permeant GnRH receptor binding drugs that assist receptor trafficking and function could be used as a novel therapeutic regimen for patients with low GNRHR expression due to congenital mutations or hormonal disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, 5300 (over 5 yrs)
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?	Experiments will be performed in mice, in which the biology of hypothalamic-pituitary-gonadal axis is very similar to humans. Our surgical models used to manipulate the reproductive system will be performed under general anaesthesia supplemented with analgesia. These surgeries are classified as moderate severity but most animals normally recover within a few days post-surgery with very mild deficits only being apparent through specific behavioural or hormone/anatomical analyses. Humane endpoints will be used to ensure the adverse effects do not go beyond the minimum required to achieve the scientific objectives. Experimental animals will be terminated in the study by accepted humane method of killing.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The signals governing neuroendocrine control of reproduction are highly complex, often involving interactions with multiple cell types in their microenvironment, and regulation by both cell-cell contact and secreted factors. Our hypotheses must be tested and refined in models where the complex environment of the intact hypothalamic-pituitary-gonadal-axis is present. We currently do not have the ability to reproduce these conditions outside an organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimize the number of animals used by employing multiplexed analysis combined with highly sensitive and information-rich detection techniques to maximise amount of information extracted from test samples, thereby reducing the numbers of animals required. Animal group sizes will be determined based on experience, previous experiments and pilot studies using power calculations whenever possible with reference to statistical readouts, so that the number

	of animals is sufficient to achieve statistically significant results. If we are unsure of what appropriate statistical tests to use we will consult statistical advice locally.
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 species of choice for these studies due to its highly characterized hypothalamic-pituitary-gonadal axis and the high degree of functional similarity to humans. The ability to genetically modify the mouse germline is superior to other mammals and the availability of probes and antibodies enable us to accurately identify relevant cellular relationships. In particular, we will use humanized animal models that accurately reflect characteristics observed in human patients, so that the observations obtained from evaluation of human specific molecules will be more precise and provide relevant information about human disease, rather than approximate models. By using more refined models we will limit off-target effects that often disturb the wellbeing of the animal, and confound data analysis. To minimize suffering we will have developed precise protocols to interrogate specific brain cell populations using vectors, which are not known to cause any adverse health effects.

<table>
<tr><td>Project 5</td><td colspan="3">Use of a natural biomaterial for hypospadias repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Biomaterial, urinary tract, hypospadias, urethra</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">2-3 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</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>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>~~Yes~~</td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">1 in 300 newborn baby boys are born with a condition known as hypospadias. This means that the hole that they urinate from is not in the correct position. There may be an associated curvature of the penis and an abnormal foreskin.

Current management in most boys with hypospadias involves surgery. In minor cases this can be achieved by one operation. However in moderate to severe hypospadias this is best achieved in two or more separate operations. The first step in these more complicated cases is to increase the amount of tissue in the area. This is achieved by attaching a skin graft to the underside of the penis. The second operation uses this skin graft to form a “urethral tube extension” so that the hole is located in the correct position.

Complications are common after surgery for hypospadias. The most persistent and difficult to manage are called “fistulas”. A fistula is a hole within the new urine tube. Fistulas can occur in up</td></tr>
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	to 20% of cases and are extremely difficult to manage. One of the reasons fistulas occur is the poor quality tissue in the area, due to the hypospadias condition. This tissue offers poor support to the repaired wee tube (urethra). This weakness leads to tension and results in complications. The lack of healthy tissue is even more noticeable in children where previous operations have failed. There is a clinical need for a material to provide extra support and behave as a "healthy tissue" substitute in order to prevent such complications. In addition such material could be used at the time of the first surgery to improve the initial success rate and reducing the need for any "redo" operations. Such a material does exist and recently has been tried in a small group of children with apparent success. However, it was not ethical to take biopsies from these cases following healing to examine how the body had reacted to the material. Therefore this information remains unknown. Our team has developed a new surgical biomaterial. The purpose of this project is to test the use of the two biomaterials for hypospadias repair in pigs and to carry out a detailed study of the outcome in order to provide us with objective information to support clinical trials.
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)?	It is anticipated that the new scaffold could revolutionise surgical treatment of hypospadias by providing extra surrounding tissues to support the repair. It is likely that the material will be useful for both complicated "redo" cases and also children undergoing their first operation. This will benefit the patient's short term and long term welfare by removing the need for multiple procedures and achieving a satisfactory outcome at a young age. From a wider social aspect, reducing the number of operations, healthcare worker hours and length of hospital stay will reduce costs to the National Health Service.

What species and approximate numbers of animals do you expect to use over what period of time?	3 live pigs will provide information on a currently available material “control group” for 3 or up to 4 months 3 live pigs will then be used to investigate the new material, using broadly the same methods (subject to any refinement of methods) for 3 or up to 4 months. This will then be repeated in a final phase, again 3 pigs in the “control” group and another 3 in the new material group. This will provide comparative and reproducible data. This will take another 3 or up to 4 months.
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?	Possible but unlikely side effects are painful urination and urinary tract obstruction. At the end of the experiment, the animals will be terminally anaesthetised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have performed all the non-animal studies we can. A small pilot study has been performed in children and was successful. To take this forward safely into routine surgical practice, we need to be fully informed of the outcome of using the biomaterial to support the tissues surrounding the urethra. This can only be performed in an animal of equivalent size and anatomy to child patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have planned our study with a minimum number of pigs. The design means that we only progress through the study when each stage is successful. We will start with 3 pigs, then another 3 and finally a further 6. We will only use all 12 animals if the study is successful.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	The operations will be performed by a paediatric surgeon who routinely performs similar operations in children and he will be assisted by a veterinary surgeon. The pig has been selected as it is most like a child in size and anatomy and hence we can

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	be assured that the procedures and the results will be relevant to surgical practice.

<table>
<tr><td>Project 6</td><td>Germline genetic inheritance and reproductive health</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fertility; Ageing; Chromosomes; Mitochondria; Cancer.</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>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 overarching goal of our research programme is to improve reproductive health in humans through the application of basic science and translational research. The proposed research consists of three areas of investigation:

1. What are the causes of the decline in fertility during female ageing?

For an embryo to contain the correct number of chromosomes, the sperm and the egg must contribute only a single copy of each chromosome at the time of fertilization. For reasons that have long been unclear, a high proportion of the eggs ovulated by women over the age of 35 years contain either too few of too many chromosomes, resulting in a high incidence of infertility, miscarriage and birth defects. In previous work with mouse eggs, we have shown that a factor known as Cohesin becomes depleted during female ageing. In this project we will investigate the mechanisms underlying how and when Cohesin is</td></tr>
</table>

	lost from eggs during female ageing. The scarcity of human eggs for research makes it necessary to use mouse eggs and ovaries for this part of the project. **2. Development of IVF-based techniques to reduce the risk of disease in children of women who carry mitochondrial DNA mutations.** Mitochondria are tiny structures within cells that produce most of the energy required for proper functioning of all cells in our bodies. Mitochondria contain their own DNA which is essential for energy production. Mutations affecting mitochondria can cause life-threatening diseases. Mitochondria are inherited exclusively from our mothers but an affected woman can produce eggs with widely varying levels of mutation. Thus, there is generally no way of predicting whether her children will be affected. We aim to address this problem by developing IVF-based technologies to produce eggs containing healthy mitochondria from a donor egg. This technique has the potential to greatly reduce the risk of disease in children of women who carry mitochondrial DNA mutations. While much of this work will involve the use of human eggs and embryos, we propose to use mouse eggs and embryos to develop and optimise the experimental techniques. **3. Fertility preservation for children undergoing cancer therapies.** It is now well established that a variety of cancer treatments can result in infertility. While adults have the possibility to store eggs or sperm before commencing chemotherapy and/or radiotherapy, there is currently little that can be done to preserve the future fertility of children undergoing cancer treatments. We aim to address this problem by using mouse testis and ovary to develop techniques for harvesting, culturing and preserving reproductive cells. This work will enable us to progress towards the development of clinical treatments to preserve fertility for children

	undergoing treatment for cancer.
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 programme of work outlined here will add to our knowledge of reproductive biology in mammals and has the potential to lead to groundbreaking treatments to reduce reproductive risk for older women and for women affected by mitochondrial DNA mutations. The research will also advance our progress towards development of clinical services to preserve the future fertility of children undergoing cancer treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse reproductive cells and tissues to understand basic mechanisms and to develop new techniques. We estimate that we will require 3200 mice during the 5 year project. A number of experiments involve the use of transgenic mice, which we will breed. However, only those with the correct genotype will be suitable for use in the 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?	The genetic modifications we propose to study are generally confined to the reproductive cells. Apart from infertility, it is highly unlikely that there will be an adverse effect on the general health of animals. All animals will be humanely killed at the end of the procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary to use mice for this programme of research because (a) The supply of human eggs is insufficient to provide conclusive answers to our research questions. (b) Robust and reproducible methods for producing human sperm and eggs in the laboratory have not yet been developed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our core approach is based on live cell imaging of individual eggs and early embryos, which has the advantage of yielding a large amount of data from individual eggs/embryos. Where knockout mice are used, we will minimise the number of mice required to maintain colonies by creating banks of frozen

	embryos.
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 because it is by far the best characterised experimental mammal. Our experiments involve minimal interventions, refined over many years, typically consisting of an injection of hormones followed by humane killing and collection of eggs and embryos prior to the transfer of these eggs to a foster mother.

Project 7	**Modelling complications of early pregnancy**	
Key Words (max. 5 words)	Pregnancy, Miscarriage, Ectopic, Maternal Health	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 overall purpose of this research is to further understanding and improve the management of the early pregnancy complications of miscarriage and ectopic pregnancy. Specifically, our aims are: 1) To determine whether Chlamydia trachomatis causes miscarriage; and 2) To identify novel drug targets for the medical management of ectopic pregnancy by targeting molecular pathways involved in placental cell growth and/or survival.	
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 primary potential benefit of this project relates to new knowledge about the causes of miscarriage and ectopic pregnancy. This information is likely to be of interest to pre-clinical scientists studying the mechanisms underpinning early pregnancy failure and ectopic pregnancy. The secondary potential benefit relates to the value of the results to clinicians, in particular gynaecologists, but also general practitioners and other health care workers. For example, we believe that a	

	combination of drugs that act independently of each other will resolve ectopic pregnancies more effectively than the current standard of care, methotrexate alone, and that this approach has the potential to significantly reduce the number of cases requiring surgical intervention. The third potential benefit will be that the data could be used as evidence for policy makers. For example, it could be useful as evidence for targeting *C. trachomatis* screening to relevant population groups. Ultimately, this work aims to help reduce the incidence of ectopic pregnancy and improve its treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use around 2000 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?	Most of the protocols we are proposing to use under this licence should cause only mild adverse effects to the animals involved and standard animal husbandry procedures, such as Ear notching and implantation of microchips should involve only slight and transient pain, with no long-term adverse effects. In all instances, animals will be monitored closely throughout the experiments and will be killed using standard humane methods where there is any evidence for harm or distress exceeding agreed severity limits. Established guidelines for anaesthesia and analgesia will be followed at all times. The first protocol (Mild severity) involves infecting female mice with a strain of Chlamydia that only infects cells lining the urogenital tract and does not appear to cause any pain or distress. We anticipate that mice infected with this strain of Chlamydia will experience a higher rate of early pregnancy failure than normal, but this is unlikely to be associated with measurable pain or distress. The protocol ends when mice are killed using standard humane methods towards the end of their pregnancies (day *14* of pregnancy) or if agreed severity limits are likely to be exceeded. The second protocol I Mild severity) involves treating normal pregnant mice with drugs that

	are predicted to induce abortion and comparing the rate of pregnancy failure in these mice with untreated mice. This model is unlikely to cause distress or harm to the mother, other than any mild adverse effects of the drugs administered at doses known to be non-toxic on day 10 of pregnancy. The protocol ends when mice are killed using standard humane methods on day 14 of pregnancy or immediately if mild severity limits are likely to be exceeded. The final protocol (Moderate severity) involves injecting the skin of an immune-compromised strain of mouse (the nude mouse) with human placental cells and assessing the ability of drugs to control the growth of the resulting cell masses. Previous studies have shown that human trophoblast cells rarely spread to other parts of nude mice, are unlikely to ulcerate, impair mobility or exceed a size likely to cause moderate harm or distress. The drugs used will be administered at doses known to be non-toxic and are unlikely to cause moderate adverse effects. The protocol ends when mice are killed using standard humane methods 4 days after drug treatment, and up to 20 days after injection with trophoblast cells or immediately if moderate severity limits are likely to be exceeded.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Pregnancy and pregnancy disorders do not exist outside the animal kingdom and pregnancy is simply too complex to model in the laboratory using currently available technology. Some useful information on relevant pathways can be acquired ex vivo using human tissue collected (with consent) from women undergoing surgery for miscarriage, ectopic pregnancy and hysterectomy for benign gynaecological conditions, and we conduct many such studies in our laboratories. However, determining causality in early pregnancy failure, as well as the efficacy of potential interventions, currently requires some experiments to be conducted in animals as it is not ethical to conduct such studies in

	humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The applicant has extensive experience of using statistics in experimental design and analysis, but formal statistical advice is available from the institute. We will minimise the numbers of animals used in this project by performing pilot studies to allow accurate sample size power calculations to be performed on the proposed experimental designs and methods of analysis.
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. We will use mice because they have the advantages of a short gestation period, the availability of genetically manipulated strains, the presence of existing models and, thanks to its widespread use as a laboratory model, much is known about implantation and embryo development in the mouse and the mouse is a well-established model of infection-related pregnancy failure. Choice of model. (Protocol *1)* Current genital infection with Chramydia muridarum is reported to alter the ability of mouse oviduct cells to transport eggs 14 days after infection but there is no information in the literature regarding long-term effects of naturally cleared, or antibiotic treated, infection with the human pathogen, Chlamydia trachomatis, on murine female reproduction. The mouse model of vaginal infection with Chlamydia trachomatis serovar F is the most refined model available at this time with which to study the effects of genital chlamydial infection on pregnancy outcome. (Protocols 2 and 3) There are currently no mouse models of tubal pregnancy as embryos are thought to degenerate rather than implant ectopically in the mouse. We will, therefore, use the mouse xenograft (human cells transplanted into mice) and fetal reabsorption models previously developed and refined by others. Minimisation of animal suffering. Drugs and other substances will not knowingly be given in doses expected to produce maternal toxicity. Animals will be monitored frequently and closely for symptoms after administration of drugs or substances, Infectious agents will be

	administered at doses aimed to cause little or no maternal harm or distress. Animals will be monitored closely throughout and where there is evidence of adverse effects approaching protocol severity limits, animals will be killed immediately by standard humane methods. Should this occur, experimental protocols will be reviewed.

Project 8	**Cytoplasmic regulation of mRNAs**	
Key Words (max. 5 words)	Gene regulation, mRNA translation, post-transcriptional control, RNA-binding protein, translational activators	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Genes encode the genetic blueprint of our bodies and must be tightly regulated to ensure that they make the proteins needed by our cells in the right time and place and in the correct amounts. Failure to regulate this process can lead to numerous diseases. Work in recent years has revealed that mis-regulation of particular steps in the pathway of synthesising proteins from genes known as “mRNA translation and mRNA stability” are important in the aetiology of a wide range of human diseases including reproductive, neurological and metabolic disorders. Our work examines how a class of regulatory proteins known as “RNA-binding proteins”, regulate the mRNA translation/stability steps and the health consequences of mis-regulation. Humans have over 1000 RNA-binding proteins which are likely to impact on many different biological processes required to develop and maintain a healthy body but to date only a few of these regulatory proteins have been studied. Thus this work has three distinct but overlapping aims:	

	1. The identification of novel regulators of mRNA translation/stability 2. Understanding the molecular mechanism of identified translational/stability regulators 3. Understanding the roles of these regulatory events in biological processes
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)?	Regulating the synthesis of proteins from genes (gene expression) is critical to human and animal health. Recent work has shown that “RNA-binding proteins”, which we study, regulate almost all aspects of gene expression are far more abundant than previously realised. Despite this, the function and mechanisms by which they exert regulation and their contribution to animal/human health remain unknown in most cases. In the short term by identifying which of these regulatory “RNA-binding proteins” control particular steps in the gene expression pathway (mRNA translation/stability) and understanding how they achieve this regulatory function, our work will provide insight into the fundamental mechanisms by which gene expression is regulated. This in important as regulating gene expression is fundamental to all aspects of life and as such dysregulation of this process is linked to the aetiology of a wide variety of animal/human diseases. Moreover, results from ourselves and others already suggest that the families of “RNA-binding proteins” to be studied are, or are likely to be, important in a number of human diseases. Therefore, in the longer term our work has the potential to prove valuable to both the understanding of specific human diseases and the identification of potential diagnostics or therapeutic avenues.
What species and approximate numbers of animals do you expect to use over what period of time?	Xenopus laevis (African claw-toed frog) 1,600 Mouse 24,100
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	The majority of frogs will be used for the collection of immature eggs after humane termination or to lay eggs. These eggs will be manipulated and fertilised to test the importance of our regulatory proteins in development and future reproduction. The majority of mice will be used for breeding. This allows us to study their reproduction and to harvest tissues/cells for further study after humane termination.

end?	Some animals will be used in experiments e.g. they may be injected with compounds, blood taken, their diet altered, tissues imaged, or their behaviour studied. This will inform us about the importance of our regulatory proteins in mammalian biology (e.g. development, reproductive function). A small number will be subject to surgical procedures to give detailed insight into why their reproduction is faulty. The severity is mild or moderate. Most animals will be humanely terminated but due to the mild nature of some of the experiments a small number may be kept alive for further study under this licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We primarily use animals to study the roles of our proteins in reproduction and development as these processes are very complex involving many cell types and organs and therefore cannot be recapitulated to any great extent outside of a living organism. However, the majority of our work uses non-animal based approaches e.g. biochemistry, cells in culture, cell-free systems, biophysics, molecular biology, yeast genetics, in silico approaches, molecular modelling where applicable. These approaches help to refine the questions that need to be asked in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals are only used when all the other approaches we use (see above) cannot answer our scientific questions e.g. what is the effect on health when a specific gene is mutated. Experimental questions using animals are refined based on data obtained by other approaches (e.g. in vitro) and pilot data are analysed to further guide experimental design and to ensure that statistic power is reached using the minimal number 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	Where applicable, Xenopus are used to explore the in vivo functions of our RNA-binding proteins-but mice are now increasingly used where Xenopus are inappropriate e.g. missing (lie gene for the protein, missing the biological process under study, or when we are aiming to establish relevance to mammalian/human health. Good experimental design and the refinement of questions using other approaches minimises the amount of animals used,

(harms) to the animals.	and our experimental design aims to utilise the least invasive approach to answering the scientific questions. Experienced a rid dedicated staff further minimises the risk of welfare issues.

<table>
<tr><td>Project 9</td><td>Regulation of ion transporters expressed in the kidney</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>High blood pressure, Salt intake, Salt excretion, metabolic syndrome</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</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>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To understand how commonly used immunosuppressive drugs induce high blood pressure and metabolic changes</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>Improved therapies for transplant patients, improved therapy for hypertension and metabolic syndrome</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>1500 mice over 5 years</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</td><td>Mild immunosuppression and hypertension. Immunosuppression potentially manifesting with infections or malignant tumours (unlikely over the short experimental period). Animals identified with</td></tr>
</table>

level of severity? What will happen to the animals at the end?	one of these or unexpected problems will be identified through monitoring behaviour, food intake and weight, and, if necessary referred to NVS for definitive advice.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are going to answer some of the questions related to this project in cell culture and using the Xenopus oocyte expression system. However, unknown pathways can only be identified in intact organs close in the pathophysiological mechanisms found in the human situation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot experiments will be conducted for a limited number (5) of animals. This will allow power calculations to minimize the number of animals used to achieve significance for our experiments. Genetically defined inbred strains of mice will further reduce variability, and thus numbers required.
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 choice of rodents is essential for both the amount of tissue needed for biochemical experiments, the similarity to human pathophysiology, the requirement for urine and blood pressure monitoring, and the availability of the desired genetically modified animals. Pilot experiments will allow us to refine experimental methods to minimize variability and maximize animal welfare. General measures: 1)Close monitoring of animals which have undergone procedures 2)Termination of experimental series where a high number of unexpected adverse effects are observed or where these affect welfare 3)Development of techniques to minimize harms where adverse effects are unavoidable.

<table>
<tr><td>Project 10</td><td>Nerve growth and regeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stimulating nerve growth and guidance</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</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>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Damage to the spinal cord that cuts connections between the brain and the limbs invariably causes paralysis and lifelong confinement to a wheelchair. Therefore, finding ways to stimulate the spinal cord such that nerves re-grow and reconnect properly is a major focus of research. Our group has pioneered studies which aim to use small electrical signals in combination with other chemical and physical guidance cues to both stimulate and direct nerve regeneration. These experiments range from observations made in a dish where cells can be made to grow in one direction or the other, to experiments combining multiple guidance cues in 3 dimensional tissue simulation models. Our discoveries have led to the use of electrical stimulation methods in the first clinical trials on human patients with severe spinal damage. The results of these trials showed significant improvements in sensory function but no improvement in motor function. Although the use of electrical stimulation in spinal research has</td></tr>
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	progressed to this near-patient level, our understanding of why nerves respond and of what electrical, chemical, physical or combined parameters are optimal is far from complete. Indeed one could argue that human clinical trials are premature because of our limited understanding of the underlying mechanisms. Our continuing work aims to provide an in depth understanding of the mechanisms used by spinal nerve cells to respond to these tiny electrical signals and to other guidance signals in combination. It will therefore play a major role in optimising the use of electrical stimulation in spinal cord repair.
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 important outcomes from this project will be a much improved understanding of what types of growth and guidance stimulating cues should be used and in what combination to promote spinal cord regeneration. This is an urgent, unmet clinical need.
What species and approximate numbers of animals do you expect to use over what period of time?	The amphibian Xenopus laevis, commonly called the African clawed toad. We inject one adult female per week to induce egg laying. We then fertilize the eggs and grow the cells from the early developing spinal cord in culture. Over a five year project we estimate 250 animals.
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?	This is a simple injection procedure and has a mild severity rating. Transient minor discomfort during injection or manually induced egg laying could occur. Our extensive experience has shown this to occur in less than 1% of animals. Females can be recycled roughly monthly and will lay many such clutches of eggs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Culture of neurons is the best way to observe dynamic aspects of nerve growth both because it allows access for detailed imaging and for the ability to manipulate neuronal growth conditions.

use non-animal alternatives	
2. Reduction Explain how you will assure the use of minimum numbers of animals	A reduction and a refinement of the procedure is the re-use of animals for egg and sperm production. Re-use for this purpose is considerably less stressful and harmful to the female than the process of importing them into the laboratory and the criteria for re-use is agreed in advance with our registered vet.
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 Xenopus spinal neurone culture system is the Gold Standard for neuronal growth and guidance studies. We use well-established hormone treatment of mild severity to induce egg release to create embryos en masse and tissue for one week of work. One male is sacrificed for the fertilisation and the sperm is stored and reused for an additional week. The cost in terms of animal numbers is approximately three females injected per week and 2 males sacrificed per month for continuous daily experimental cell cultures. Females regenerate their eggs over a few weeks and readily produce large numbers of eggs when re-used not less than 4 weeks but typically 12 weeks after a previous injection. Re-use greatly reduces the number of animals that we kill. A colony of 36 females can produce a continual supply of eggs weekly for several years through careful re-use of individuals. We only use laboratory-bred Xenopus (currently either supplied by NASCO Inc. or bred in-house), which have a good health status, are content and more used to being handled. As a consequence, they thrive better in the laboratory environment and produce eggs and sperm of better and more reliable quality (more significant experimental data and reduction of animals required). In addition, groups of licence holders organise their work around specific days when frogs are laying to make the most of the egg production and sperm availability that day thereby saving on the overall use of frogs. This process is coordinated in advance by the registered animal technician.

<table>
<tr><td>Project 11</td><td>Mechanisms of meiosis and causes of aneuploidy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Meiosis, oocyte, aneuploidy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</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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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>All animal life starts with the fertilization of an egg by sperm. During fertilization, the genetic material of the mother and the father are united. Genetic material is stored in the form of chromosomes. The egg contains half of the chromosomes of the mother, and the sperm contains half of the chromosomes of the father.

Sometimes, the embryo may obtain the wrong number of chromosomes. This will most frequently result in pregnancy loss and infertility. In other cases, the embryo may be viable, but it will suffer from congenital disorders such as Down's syndrome, in which the embryo has three instead of two copies of chromosome 21.

From previous work we know that embryos frequently obtain the wrong number of chromosomes, because the egg does not contain exactly half of the chromosomes of the mother. This happens even more frequently, when women get older and is called the “maternal age effect”.

To understand why eggs frequently have the wrong</td></tr>
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	number of chromosomes, we need to investigate how they develop. An egg develops out of a progenitor cell, the oocyte, which still contains the full number of chromosomes. To become a fertilizable egg, the oocyte has to eliminate half of the chromosomes into a small waste cell that is called a polar body. This frequently does not work reliably so that the wrong number of chromosomes stays in the egg. Our aim is to understand, how the chromosomes become prepared for elimination into the polar body, and how the machinery is working that distributes the chromosomes between polar body and egg. This machinery is called the microtubule spindle and consists of protein fibres which separate the chromosomes. If the spindle is abnormal, chromosomes cannot be separated accurately.
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)?	Our work will shed light on how the spindle is organized and how it works in mouse eggs. It will also improve our understanding of how other protein fibres, which are called actin filaments, help to ensure that the embryo obtains the correct number of chromosomes. Actin is for example important to position the spindle accurately in the egg before it separates the chromosomes and to ensure that only one sperm fertilizes each egg. Finally, our work will shed light on why eggs from older women are more likely to obtain the wrong number of chromosomes. To this end, we will compare eggs from young and old mice and test which defects cause eggs from older women to develop abnormally. The work suggested in this project licence application will help to build up knowledge to improve methods for diagnosing and treating causes of infertility in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	11250 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	All experiments in this licence will be carried out *in vitro* on isolated oocytes, fertilised or unfertilised eggs as well as very early embryos (during the first days of division, before the embryo implants into

level of severity? What will happen to the animals at the end?	the uterus). To obtain oocytes, eggs or early embryos, we will kill mice with a Schedule 1 method and isolate the ovaries where oocytes are stored. In some cases mice may be killed by a Schedule 1 method to obtain other types of cells such as sperm to carry out *in vitro* fertilisation experiments. In some mice, we will remove or *knock out* the genes that might be important in the oocyte, and observe how this affects the development of the egg or the embryo, respectively. We will primarily remove genes only in oocytes, but not in other tissues. The removal of genes in the oocyte should not affect the well-being of the animals, but only affect their fertility. We will also engineer mice so that proteins which are particularly important in the oocyte are labelled and can be detected on microscopes. The vast majority of mice will have mild or no signs of ill health. Since we intend to compare oocytes from young and old females, we will need to keep some females until they are more than one year old. These females are more likely to signs of ill health such as a gradual body weight loss, marked piloerection, react subdued even if provoked or show little peer interaction. Mice will be killed by a Schedule 1 method if they reach 20% weight loss compared to the start of experiment or exceed 3 clinical signs indicating moderate severity. Less than 10% of all animals on this project licence are expected to reach the moderate level of severity. All mice will be killed at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, functional oocytes cannot yet be derived from stem cells so that we need to isolate oocytes from animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be kept to the minimum required for breeding purposes. Strains that are not currently required will be cryopreserved. We will mostly use live cell imaging approaches. These have the advantage that only a small amount

	of biological material is required. We will always employ statistical tests to determine the minimum number of animals that is required to obtain statistically significant results. We successfully established methods that now allow us to deplete many stable proteins in the oocyte without generating a knockout mouse. This means that we do not rely on the generation of knockout lines anymore for many genes, which greatly reduces the number of animals that we need for our experiments.
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.	We will work with mouse oocytes, because they are the best model for human oocytes that can be studied with state-of-the-art molecular cell biology and genetic tools. It is important to study the development of the egg in a mammalian model system, because eggs in other animals, such as frogs and birds, strongly differ from mammalian eggs because mammals develop inside the body. Where possible we will block gene function only in the oocyte so that the wellbeing of the animal is not affected. Those mice that we will keep until they are more than one year old to investigate how maternal age affects oocyte quality will be monitored carefully by daily inspection. Mice that are beginning to develop clinical signs will be killed and analysed before they reach the moderate severity limit whenever possible.

Project 12	**Renal inflammation, fibrosis and repair**	
Key Words (max. 5 words)	inflammation, fibrosis, repair	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Chronic kidney disease affects many patients and is characterised by inflamed and scarred kidneys. Chronic kidney disease often progresses to end stage kidney failure that is uniformly fatal without dialysis or kidney transplantation. Diabetes remains the commonest cause of kidney failure in the developed world. Recent work has suggested that kidney scarring can regress although this may take many years. This project will use experimental models of diabetic kidney disease and kidney obstruction that exhibit injury and scarring as well as some reparative tissue remodelling as these are relevant to human disease. We will use unbiased scientific approaches to understand which particular cells and mediators are iniolved in the progression and resolution of kidney inflammation. We also aim to identify molecules that can be used to assess the extent of inflammation, injury or repair of a kidney as these may be used to assess the response of patients to novel therapies and monitor progression or resolution of disease.	

	In addition, we wish to test the potential of novel therapies suggested by the work of ourselves or other investigators in these translationally relevant models of renal inflammation and scarring to determine if they are of benefit. This preclinical assessment of potential therapies will generate a platform for future clinical trials in patients.
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 may produce results that are highly relevant to human disease. Firstly, the work will provide increased understanding of the fundamental biological mechanisms underlying detrimental kidney inflammation and scarring and beneficial kidney remodelling. Secondly, it will explore the utility of novel therapies that may be translatable to patients. For example, we know that various growth factors can drive the proliferation and survival of cells that generate scar tissue. We will determine whether drugs that block the actions of these growth factors can retard or improve fibrosis in the obstructed or de-obstructed kidney. Some of these drugs are already licensed for use in patients with non- renal diseases and our experiments may suggest that patients with injured and scarred kidneys might benefit from treatment with these drugs. This would require a clinical trial of these drugs in, for example, patients who are left with chronic kidney disease despite successful relief of kidney obstruction caused by prostate disease.
What species and approximate numbers of animals do you expect to use over what period of time?	2000 adult mice and 400 adult rats will be used 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?	These experiments will involve the induction of diabetes and hypertension as well as surgical procedures (the removal of a kidney or obstruction/de-obstruction of a kidney). Potential adverse infections include excess weight loss, infections and post-surgical discomfort. Diabetic animals will be carefully monitored and insulin will be used to prevent excessive weight loss. Blood pressure will be monitored so that it is not excessive.

	Surgical procedures will be undertaken by experienced researchers using sterile techniques and analgesia given to minimise discomfort. Animals will be monitored for any distress and procedures requiring blood sampling such as assessing glucose tolerance will be undertaken according to established guidelines. At the end of experiments animals will be culled and tissues etc harvested for analysis and stored for potential use in further studies. The named veterinary surgeon will be consulted if infections or health problems are problematic and animals showing signs of continuing disease or distress 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	The cause and progression of renal disease is complicated with many cells and mediators contributing to the evolution of inflammation, fibrosis and tissue remodelling. Attempts have been made to use 2 or 3- dimensional cell culture models to explore these processes but, although useful, these models do not reflect the complexity of disease within the kidney. The experimental models of diabetic nephropathy that most closely resemble human disease involve both high blood sugars and a high blood pressure and the latter simply cannot be replicated in a cell culture environment. Our proposed models of diabetic nephropathy, and kidney obstruction/de-obstruction do reflect the dynamic and multi-faceted nature of kidney disease in humans disease and are appropriate for furthering our understanding of disease processes and testing novel therapies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are experienced in the chosen disease models. tatistical analysis of our previous studies indicate that experimental groups of 6-10 animals are suitable to generate robust

	data. Despite this we will undertake power calculations before experiments whenever possible to calculate group size. We will include appropriate control groups so that experimental data is scientifically interpretable. We will carefully store tissue, urine and blood samples from experimental animals such that additional future studies can be undertaken on this archived material whenever possible. This material will be made available to other research groups on request so that additional experiments involving animals will not be required.
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.	We have chosen models of diabetic nephropathy, and kidney obstruction/de-obstruction that are analogous to human disease. Mice will be used in light of the ready availability of genetically-modified mice available for incisive dissection of disease processes. We will also employ a rat model that closely mimics moderately advanced human diabetic nephropathy. Animal suffering will be minimised by regular monitoring by trained staff, optimisation of blood pressure by remote radiotelemetry monitoring to reduce animal handling, appropriate use of insulin treatment to prevent excess weight loss. In models of diabetic nephropathy, serial measurement of urine rrotein levels will allow us to assess the severity of nephropathy and terminate the experiment as soon as possible after the onset of significant disease or after the efficacy of therapies is demonstrated. Our model of diet- induced hypertension offers the possibility of modulating hypertension to the minimum scientifically required. Mice tolerate the surgical kidney obstruction/de-obstruction model well and pain killers will be administered before and after surgery to limit discomfort.

Project 13	**Investigation of gonadal development**	
Key Words (max. 5 words)	Fertility, ovary, testis, embryo	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Successful reproduction requires the optimal development of sperm and eggs, but we still know relatively little of what regulates their development, or how they can be affected by an adverse environment. The work here will investigate how germ cells are formed, and how normal development occurs, in both males and females. We will also examine how that development can be affected by exposure to detrimental effects such as chemotherapeutic agents. Much of the work we do uses tissue culture techniques, with animals required only to obtain material for the culture work: experiments here will also seek to extend the capabilities of such culture techniques, so that they can be of greater use in the future.	
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 project will increase our fundamental knowledge of germ cell development. Advances in this area will help understand potential causes of infertility. Work examining detrimental external effects such as to chemotherapy drugs will be of direct benefit to cancer patients. Recent decades have seen steadily growing survival rates for all cancer patients, and, for younger patients of reproductive age, the potential loss of	

	fertility is a worrying side effect of such treatment. Our work aims to determine exactly how such drugs affect the developing sperm and egg, with a longer term aim of investigating how such damaging effects can be blocked, or at least ameliorated.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, rats; around 8,000.
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 proposed work will have only mild adverse effects, for example, injection of substances, or breeding of genetically modified mice that have affected reproduction only. In about 2/3 of cases, animals will have no invasive procedure carried out while they are alive. The maximum severity is classified as moderate, which relates to surgery with analgesia in a small number of animals. At the end, animals will be killed, and they or their offspring observed/examined or their tissue cultured.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Much of the proposed work uses tissue culture techniques, replacing the need for many in vivo procedures. However, that work still requires the use of mice, including genetically modified animals, for the collection of tissue for culture, and to examine whether cultured eggs or sperm are able to fully support fertilisation and subsequent embryonic development. In vivo studies are also used to confirm in-vitro findings.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The regular use of culture techniques allows for a continual reduction in animal numbers. Experiments will use the smallest number of animals required to produce statistically significant results, with the use of insufficient numbers of animals a waste, as it would not allow robust, scientific conclusions to be drawn. For any one experiment, the number of animals needed depends on the magnitude of the expected effect: prior experience usually allows us to determine this number.
3. Refinement Explain the choice of species and why the animal model(s)	The work uses rodents (mice and rats), with protocols that are well-established and well-refined. Most transgenic lines will have little discernible phenotype, and most work will involve collection of post-mortem

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.	material for analysis or culture. Where surgery is required, work will be carried out quickly, and animals closely monitored to minimize the risk of pain and suffering. Where we see evidence of unreasonable harm, animals are humanely killed.

<table>
<tr><td>Project 14</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>
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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>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 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.</td></tr>
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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, these 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..

<table>
<tr><td>Project 15</td><td>Receptor functions in development and infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Receptors, fertility, pathogens, vaccines.</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>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objective of this project is to investigate the function of extracellular protein interactions identified using in vitro protein interaction methods. We are interested in cell surface and secreted protein interactions that are important for the pathogenesis of certain infectious diseases and normal developmental processes, particularly fertilization. We will be studying pathogens which affect human health, and for which no vaccines are currently available or licenced. Our work on developmental processes is aimed at obtaining a basic understanding of how fundamental biological processes work; for example, we are interested in understanding more about how sperm and eggs recognise each other.</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</td><td>The work we are performing on understanding how certain infectious diseases can interact with human cells may lead to the development of new vaccines for both human and animal use. We work on cell surface and secreted proteins which, because they</td></tr>
</table>

project)?	are exposed to host antibodies, are inherently very attractive vaccine candidates. The work described in this project will provide new approaches in the preclinical development of vaccines for human diseases such as schistosomiasis, babesiosis and malaria.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using mice as a model organism. We anticipate that we will use 2,500 per annum and the project is expected to last for 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?	Some of the protocols described in this project are classified as “moderate”. The adverse effects that we are expecting include possible ulceration at vaccination sites and symptoms that result from infection challenges, which will be necessary to test the efficacy of vaccine candidates. All animals will be humanely culled by a Schedule 1 procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals to make antibodies which work well in our protocols since non-animal alternatives typically result in antibodies whose properties are not adequate for our work. We need to use animals in our preclinical vaccine screening approaches since there are few or no adequate *in vitro* infection models available for the pathogens that we wish to study. A vaccine candidate that showed efficacy in an animal model would also lend significant weight to its candidature for progression towards a human vaccine.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have recently developed a method of making more antibodies at one time in fewer animals. For the infection challenge models, we will seek advice from other scientists who are developing appropriate infection animal models to ensure that we are using the most recent and reliable pathogen strains and routes of infection. We will use superovulation to increase the number of eggs that we can obtain per mouse, which reduces the total number of animals required.

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.	All the research outlined in this project uses the mouse as an animal model. Mice are a suitable model for these studies because they are a mammal, and almost always contain a recognisable counterpart to any human proteins that we identify in our *in vitro* screens. Consistent with this, many pathogens that infect humans also infect mice with similar pathogenic outcomes (e.g. *Shistosoma mansoni*, the parasitic worm that causes schistosomiasis in humans) and so they can be used as an appropriate model for preclinical work. Mice have a long history of making important contributions to the understanding of human biology and many valuable resources such as gene-deficient mice are available that enable us to make scientific advances with an increased confidence of correctly interpreting the outcomes of experiments designed to discover new treatments and therapeutics to improve human health.

Project 16	**Oocyte epigenetics: mechanisms and effect of diet**	
Key Words (max. 5 words)	epigenetics, methylation, oocytes, embryos, diet	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	The egg is the foundation of life, developing from a single fertilized cell to a multicellular organism capable of independent existence. The egg carries with it the genetic information from the mother, but it also provides much more for successful development of the baby. It provides the instructions that allow genes to be turned on and off at the right times during the early stages of development of the embryo. Some of these instructions are in the form of 'epigenetic' information – epigenetic refers to how genes are marked by natural chemical tags. It is suspected that where these tags are normally placed in the DNA of the egg or embryo can be influenced by factors such as what we eat, whether we suffer from conditions like diabetes, or by procedures needed to treat infertility. Abnormalities in these epigenetic tags are potentially harmful, because they can persist in the developing embryo and cause genes to be active or silent wrongly. In this project, using newly development methods that can map chemical tags with great precision, we shall ask whether the	

	epigenetic landscape of the egg and embryo is altered by the diet of mothers, how this happens, and how many such mistakes are passed on and cause a problem to the developing baby.
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)?	Understanding the extent to which the epigenetic information in the egg or embryo can be compromised is vitally important, because of the possible long-term effects on health, especially in view of the increased incidence in mothers of diseases like obesity and diabetes, or the increased use of infertility treatments. Knowledge gained from this project should help inform health guidance and techniques used in infertility treatments. Our work will also advance the scientific understanding of how the egg is epigenetically marked in preparation for fertilisation and embryo development.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice and we expect to use about 1700 mice each 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?	Genetically altered mice to be used in this study will be selected to remove genes only in eggs, therefore, there is not likely to be an impact on the health of these animals. Dietary manipulations, such as high fat diets, are expected to induce obesity in mice, but the progression of obesity will be carefully monitored and the duration of the diets will be such that harmful complications of obesity are avoided. Damaging the pancreas to eliminate insulin producing cells is expected to cause problems in the control of blood sugar levels and the development of diabetes. These animals will be carefully monitored and their diabetes controlled by administration of insulin if necessary. At the end of the studies, mice will be killed using the most humane methods to collect eggs, embryos and other tissues in which to investigate effects on epigenetic tags.
Application of the 3Rs	
1. Replacement	We need to use animals for this study because it is not possible yet to develop mammalian eggs in the

State why you need to use animals and why you cannot use non-animal alternatives	test tube, nor to reproduce the earliest processes in mammalian embryo development, at which time the epigenetic tags are probably most sensitive to adverse diet and other factors, in purely cell-based systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have been able reduce the numbers of animals needed for these investigations because we have been able to develop highly sensitive methods for profiling the location of epigenetic tags in very small numbers of cells.
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.	We use mice in these studies because in this species we understand the most about where and how epigenetic tags are placed in the DNA, and because we are able to follow the fate of epigenetic mistakes during development in this species in a way that is not possible in other mammals, especially humans. We believe that the processes that put epigenetic tags in place in the mouse are very similar to those in humans, so the mouse is a very informative model. Harm to animals is minimised by using sterile conditions, anaesthetics, humane methods of killing, and by targeting genetic mutations to the cells of interest (eggs) to avoid whole-animal suffering. The welfare of each animal is monitored daily by animal care staff, veterinary staff and/or scientists. If, in rare circumstances, an animal has an unexpectedly severe response to a drug or operation, or where an infection develops, treatment is given where possible and, if necessary, the animal is humanely killed.

<table>
<tr><td>Project 17</td><td colspan="3">Mechanism and functional role of calcium signals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Calcium; cell signalling; fertilization; transgenics</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 section 5C(3)</td><td colspan="3"><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>Yes</td><td></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">Calcium regulates diverse physiological processes, including fertilization, insulin secretion by the pancreas, heart contraction, and operation of the immune system. Identifying and characterizing how calcium does this could lead to development of new drugs and therapies. One particularly important process where calcium signals play a key role is during fertilization when the sperm activates the egg to develop into an embryo. Having in our previous work identified the protein in the sperm that does this as phospholipase C zeta (PLCzeta), in this project we will seek to uncover important new information about the mechanism of action of PLCzeta and its link with human infertility using animal models. The calcium mobilizing messenger nicotinamide adenine dinucleotide phosphate (NAADP) regulates many important processes but many questions remain unresolved regarding its precise mechanism of action and physiological role. Recently we identified the two-pore channels (TPCs) as integral components of the target receptor for NAADP. A key goal of this project is to understand how TPCs</td></tr>
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	mediate NAADP-regulated calcium release and its diverse effects within the body. Another goal is generation of transgenic versions of other mammalian species besides mice which would have important implications for medical research. We have been pioneering ways to introduce transgenes into sperm and in this project we plan to explore the feasibility of using this approach to study sperm function and as a way of generating transgenic animals. We will also explore the potential of new methods of *in vivo* gene editing in the fertilised egg as a way to generate knockout versions of other mammalian species besides mice. This could both help refine transgenic techniques and the numbers of animals used by them.
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 expected to make a major contribution to our understanding of the molecular mechanisms underlying the role of calcium signalling in processes as diverse as fertilization, pancreatic secretion, cardiac function, immune function, and neurobiology. By identifying the mechanism of action and physiological role of PLCzeta it should lead to new ways to diagnose and treat certain types of male infertility, and identify targets for new contraceptives. By identifying the molecular components of key intracellular calcium signalling pathways it should lead to the identification of new drug targets for treatment of conditions such as heart disease, obesity and diabetes, and disorders of the immune and nervous systems. By developing genetically modified animals with defects in key calcium signalling pathways, this project will generate important model organisms that will play a vital role in our elucidation of the functional role of these signalling pathways in the body.
What species and approximate numbers of animals do you expect to use over what period of time?	Maximum numbers to be used annually: mice (4100); guinea pigs (200); rabbits (100); zebrafish (600); Tetraodon (100). However, numbers should be substantially less, as some strategies may not be employed or rarely employed once optimum strategies are determined**.** Most animals will be used for breeding and maintenance.

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 animals will be wild type and knockout mice that will be bred and maintained; the vast majority will have a mild phenotype but we may also study those with a moderate phenotype. Several procedures (e.g. *in vivo* gene transfer, artificial insemination, implantation of embryos) will involve surgery which could cause some post-operative pain. Other procedures (e.g. superovulation, capacity for angiogenesis) will involve injection of substances that could cause minor and transient discomfort. Fish eggs and sperm will be extracted via a mild procedure. Metabolic analysis may involve change of diet/fasting that may lead to weight loss/gain. Analysis of muscle/cardiovascular function may also lead to weight loss. Analysis of immune responses will involve treatment with infectious agents (e.g. flu virus) that may lead to moderate suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use a variety of *in vitro* approaches, such as cell culture, biochemical systems like the sea urchin egg homogenate, and also use of human material, e.g. sperm. Although much can be learned about cellular processes by studying gene function *in vitro*, this approach has major limitations for understanding how genes work *in vivo*. Animal models will complement human studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental design has been considered to reduce and refine the procedure such that numbers of animals are the minimum that will provide statistically valid data for analysis based on previous experience and power analysis. The development of electroporation/viral vectors to introduce transgenes or knock down gene expression and new *in vivo* gene editing methods offer a potential new ways for reducing animal numbers compared to standard transgenic approaches.
3. Refinement	The use of transgenic and knockout mice has revolutionalised biological research by allowing the

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.	dissection of gene function in a living mammal. As such, mice will be the primary species being studied in this project. However, for many areas of research, mice are far from being the optimum experimental species. Guinea pigs and rabbits are particularly important for studying cardiac and pancreatic function. Zebrafish and tetraodon are important experimental organisms for studying embryo development. Pain following surgery will be minimised by application of analgesics, and adverse effects carefully monitored with clearly defined procedures determining steps to alleviate suffering and identify humane end points.

<table>
<tr><td>Project 18</td><td colspan="3">Papillomavirus Life-Cycle Regulation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Papillomavirus, Epithelium, Tropism, Warts, Regression.</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>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 colspan="3">Our work aims to understand how the papillomavirus life-cycle is regulated at different epithelial sites, and how changes in the cellular microenvironment can regulate and suppress viral gene expression. Our goal is to understand why HPV-associated cancers are restricted primarily to certain ‘susceptible’ sites in the body. In addition, we aim to understand how the immune system controls the spread of HPV disease and can in in some cases lead to disease-regression.</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">Papillomaviruses cause a number of problematic diseases in humans including recurrent respiratory papillomatosis, which can cause breathing difficulties in children, and cervical cancer, which can result from persistent infection by ‘high-risk’ types. How the course of disease is regulated depends both on the immune environment and on the specific epithelial site where infection occurs. Our ultimate goal is to develop clear approaches to</td></tr>
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	control and eliminate recalcitrant papillomavirus infections in humans. This will have a major impact for individuals suffering from the problems of HPV infection.
What species and approximate numbers of animals do you expect to use over what period of time?	The work will make use of a recently identified mouse papillomavirus (musPV), which can produce productive lesions in immunocompromised mice. Because we are looking at different immune and genetic backgrounds as well as different epithelial sites, the questions that we are asking are quite complex. As a result, we expect to use up to 2000 mice during the course of the study. In addition we will use a low number of other animals (i.e. rats and guinea pigs (10 of each approx)) as controls. Although these can be infected by musPV, they do not develop disease. A papillomavirus that infects rabbits (ROPV) will be used in a small number of cases to establish whether information generated in mice is likely to extend more generally to the papillomavirus group as a whole. A maximum of 30 rabbits will be used for this work.
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?	Papillomaviruses cause benign epithelial lesions that are self-limiting in immunocompetent hosts. In immunosuppressed animals the lesions can spread, but will always be restricted to the epithelium as papillomaviruses are epitheliotropic viruses. Because of their precise epithelial tropism, papillomavirus-induced lesions are straightforward to monitor visually, and any adverse effects can be addressed rapidly. At some epithelial sites, such as the oral cavity, extra care will be taken when monitoring lesion size. Similarily, immunosuppression regimes are expected to facilitate an increase in lesion size in infected animals, which will also warrant closer monitoring. The effects of HPV infection are likely to be mild or possibly moderate in some cases. If adverse effects become more serious than this the animals will be killed by a schedule 1 method.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The lab already makes extensive use of tissue culture and organotypic raft culture approaches to study the biology of papillomavirus infections. We are in addition very active in the analysis of virus expression and virus protein function in clinical specimens, and are using these to develop our hypothesis as to how HPV interacts with the epithelial cells that it infects. We cannot however mimic particular epithelial sites in the raft model and cannot properly investigate interactions with the immune system using these methods. As a result we will use the animal models to answer questions that cannot be properly addressed using other approaches in order to produce a more complete picture of how papillomaviruses function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise animal numbers, the project will be run in a stepwise fashion, and will aim initially to understand how papillomavirus/epithelial tropisms are controlled, and how epithelial site regulates viral gene expression. Once we have a better understanding of this, we will consider the different immune cell backgrounds and also the local microenvironment as modulators of viral gene expression. A final part of the project will look at papillomavirus backgrounds in relation to genetic susceptibilities. By following a cautious approach, we expect to limited unnecessary animal usage. Although we indicate the use of 2000 mice over the course of 5 years, it is likely that the actual numbers used will be much lower than.
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.	Previously, the most appropriate animal model for the study of papillomavirus infections was the rabbit, and in our past studies we have made use of the Cottontail Rabbit (CRPV) and Rabbit Oral Papillomavirus systems (ROPV). These models were difficult to use, and we found that the rabbits often suffered significant weight loss upon immunosuppression. The identification of a mouse papillomavirus has been difficult to achieve, but recently a mouse papillomavirus has been described in the literature and has been reported to produce typical papillomas in immunocompromised

	mice. As a result we will now move our studies to the mouse model. Because the mouse papillomavirus is evolutionarily distinct from the papillomavirus types found in humans, we will carry out a limited number of comparative experiments in rabbits using the ROPV system. ROPV has some similarities in life-cycle organisation to the papillomavirus types that cause important disease in humans. By using this combined approach however, coupled with the analysis of clinical material, we envisage that we can now confine the majority of our future work to mice. As mice are a well understood laboratory host animal, we expect to be able to minimise any adverse effects during our experiments.