Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 24

Projects with a primary purpose of: Translational Research – Human Urogenital/Reproductive Disorders

Project Titles and keywords

1. **The pathophysiology of endometriosis**
    - Endometrium, Endometriosis, macrophage, nerves

2. **Mechanisms of Uterine Physiology and Pathology**
    - Endometrium, bleeding, menstruation, fibroids

3. **Molecular factors affecting sperm production and function**
    - Fertility, reproduction, sperm, sex ratio

4. **Understanding and treating pregnancy related disorders**
    - Pregnancy, preterm labour, parturition, uterus, cervix

5. **Vitamin D and immune function**
    - Vitamin D, Colon, pregnancy, placenta, inflammation

6. **Precursor cell therapies for kidney diseases**
    - Kidney Cell Mouse Therapy Scanning

7. **Improving organ function in kidney transplantation**
    - Renal, transplant, therapy, regenerative

8. **Rodent models of kidney disease**
    - Rodent models, kidney disease

9. **The effects of aging on tissue injury and repair**
    - Kidney, acute injury, chronic fibrosis

10. **Endometrial function and associated disorders**
    - Uterus, steroid, inflammation, repair, endometriosis

11. **Optimising ovarian tissue transplantation by improving vascularisation**
    - Fertility preservation, xenografting, nude mouse, human ovarian tissue, transplantation

12. **Mechanism of bladder cancer and translation**
    - Cre-LoxP, carcinogen, FGFR3, CXCR2, cell free DNA

13. **Optimisation of drug efficacy and safety in bladder cancer**
    - Cancer, chemotherapy, toxicity

14. **PKCα and vascular calcification in kidney dysfunction**
    - PKCα, Vascular calcification, Chronic kidney disease

15. **Decellularised biomaterials for homologous use in urinary bladder auto-augmentation**
    - biomaterial, urinary tract, detrusorotomy, bladder

16. **Modelling therapies for renal malformations**
    - Kidney, ureter, bladder, malformation, therapy

17. **The Causes and Treatment of Rejection of Kidney**
    - alloimunity, inflammation, tissue scarring, kidney disease

18. **Biochemical pathways in Renal Fibrosis**
    - Renal, kidney, fibrosis, therapeutic

19. **Regenerative medicine therapy for renal injury**
    - Regenerative medicine, cell therapy, kidney, injury

Project 1	**The pathophysiology of endometriosis**	
Key Words (max. 5 words)	Endometrium, Endometriosis, macrophage, nerves	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Endometriosis is a chronic inflammatory disorder that occurs in 10% of reproductive age women. It causes chronic pelvic pain and infertility. It is the growth of the lining of the womb outside of the womb, usually in the pelvic cavity. These growths of tissue are called endometriosis lesions. The objective of the project is to understand how interactions between cells in lesions cause the symptoms of endometriosis in order to determine new methods of treating the condition.	
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 estimated that 1.5 million women in the UK suffer from endometriosis. Annually the condition costs the UK £11.7 billion and this is due to healthcare costs and lost workdays. Currently the only treatments are surgical removal of lesions or hormone suppressive therapies – both have unwanted side-effects and symptoms commonly come back. We know from interacting with patients that women desperately want new alternatives to current options. Specific cells of the immune system, called macrophages are thought to play a key role in encouraging tissue growth and the growth of new blood vessels into endometriosis lesions. The project aims to understand more about the role of the macrophage in endometriosis, how the lesion environment regulates macrophage function and how macrophages interacts with new nerve fibres that	

	grow into endometriosis lesions. By using the animals proposed in this application we will learn how macrophages in lesions affect endometriosis symptoms on a physiological level by assessing the behaviour of our animal model. Ultimately, the scientific knowledge generated in this project will contribute to the development of new treatments for endometriosis that target macrophage function or the interactions that macrophages have with other cell types in endometriosis lesions.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the five years covered by this licence we plan to use a maximum of 6000 animals for breeding and a maximum of 3000 in experimental studies
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?	Endometriosis is thought to develop due to the spread of fragments of the lining of the womb into the pelvic cavity during a phenomenon known as 'retrograde menstruation'. Mice do not usually menstruate or develop endometriosis but by administering hormones they can be stimulated to undergo a process very similar to human menstruation. This material can be collected and used to induce endometriosis by introducing it into the pelvic cavity of recipient mice. The animals show no visible signs of pain or distress during bleeding or endometriosis development, but some changes in their behaviour to certain stimuli can be detected. New treatments for endometriosis can be identified and tested in this model. An intervention will be discontinued if the mice show any visible signs of pain or distress that cannot be controlled with pain relief. At the end of experiments mice 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	Endometriosis is a complex disorder that only develops in humans and some high primates. It involves the growth of the lining of the womb in the pelvic cavity and this kind of interaction is very difficult to recapitulate using isolated cells grown in a dish. The growth of endometriosis lesions generates complex physiological effects that involve the central nervous system; it is therefore necessary to model this disorder in an animal. Isolated cells and experiments on human tissue biopsies will be used wherever possible. To avoid the use of primates we will use mice to model endometriosis.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully calculated the number of animals required to produce optimal experimental results. To reduce natural variation between animals we will use mice that have been in-bred.
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.	Lower experimental organisms such as flies, frogs or fish do not have a womb. Mice have a similar womb structure to women that responds to hormones so we can stimulate it to undergo a process like menstruation. This material is collected and introduced into the pelvic cavity of recipients. This more closely mirrors the process by which endometriosis develops in women and is a significant Refinement on previously published models. It also allows the investigation of the role of the womb lining or pelvic cavity in the development of endometriosis. Mice with genetic alterations that allow the depletion of macrophages will specifically allow us to investigate the role this cell plays in the disorder. Mice will be administered pain relief before any surgical procedures to minimise suffering. Anaesthesia will be used for surgical procedures. Surgeries that will result in moderate severity include removal of ovaries and injection of womb lining into the pelvic cavity to induce endometriosis. Animals will be housed in accordance with UK home office guidance to maximise their environmental welfare

Project 2	**Mechanisms of Uterine Physiology and Pathology**	
Key Words (max. 5 words)	Endometrium, bleeding, menstruation, fibroids	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To define how the womb functions during a period and develop new treatments for abnormal bleeding (e.g. heavy or irregular periods).	
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)?	Abnormal uterine bleeding (AUB) is common and debilitating, affecting 20-30% of pre-menopausal women. Over 800 000 women seek treatment annually in the UK due to a significant reduction in quality of life. Tablet based treatments are available, but many women have surgery due to treatment failure or side effects. Each year, approximately 28 000 women undergo surgery for menstrual bleeding problems. Surgery introduces risk of organ damage, bleeding and infection. There is a clear unmet clinical need for better tablet treatments for AUB but these cannot be developed until we fully understand how the endometrium works in women with normal and abnormal, heavy periods. At the time of a period the lining of the womb (the endometrium) is shed, leaving an "injured" surface. This must be repaired efficiently, to allow the possibility of future pregnancy. Our previous studies in human tissue and cells have identified that hypoxia (low oxygen levels), inflammation and hormones are likely to have important roles in these processes. Aberrations in endometrial breakdown and repair are	

	likely to lead to heavy or irregular menstrual bleeding. Conditions, such as fibroids (benign tumours of the muscle layer of the womb), or contraceptives can also cause AUB, but the mechanisms involved remain undefined. Our research will increase our understanding of how the womb functions during normal and abnormal menstruation. They will also allow us to progress the development of new treatments for women with abnormal bleeding.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the five years covered by this licence we plan to use approximately 9900 mice
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?	Mice do not normally menstruate but with removal of their ovaries followed by administration of hormones they can be stimulated to undergo a process very similar to human menstruation. They show no visible signs of distress or pain during bleeding. This model allows us to assess endometrial breakdown and repair as well as to measure the amount of bleeding. We will administer medications to mice to assess their effects on menstruation. Alternatively, we will give a high fat diet to assess the impact of body weight on menstrual blood loss. Assessment will be carried out by removal of the womb and examination in the laboratory or by imaging the womb of mice. Studies will be discontinued in any mouse that shows signs of pain/distress that cannot be controlled with pain relief. At the end of experiments all mice 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 human endometrium is a multicellular structure with a specific architecture that affects its function. In vitro experiments provide limited data due to a lack of cell-cell interactions and loss of normal architecture. This affects processes key to endometrial function, e.g. hypoxia, inflammation, new blood vessel formation. Previous study of human tissue directs and focuses our animal studies, and will continue to do so throughout this project. However, definitive studies of the mechanisms involved cannot be carried out on human subjects. These experiments require an appropriate in vivo model, of which mice are the species of choice.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise animal numbers by using inbred strains and litter-mate controls where possible to reduce intra-animal variability. Careful experimental design, e.g. randomised block design, will increase precision. A statistician has been consulted to optimise experimental design.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For studies in rodents, the species of choice is the mouse. Mice have a uterus with a similar structure to women, e.g. an endometrial lining that covers different cell types (a secretory epithelium and multicellular stroma) surrounded by a muscle layer (myometrium). The availability of mice with genetic alterations will provide a key refinement to studies on endometrial function at menstruation. The menstruation model we use has been refined to limit the surgery necessary and maximise success (e.g. using mice >6 weeks to ensure reproductive maturity). Mice will be administered prophylactic analgesia before surgical procedures to minimise post-operative pain. Appropriate anaesthesia will be administered for surgical procedures (e.g. isofluorane inhaled anaesthetic). Animals will be housed in accordance with UK home office guidance to maximise their environmental welfare.

<table>
<tr><td>Project 3</td><td colspan="2">Molecular factors affecting sperm production and function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Fertility, reproduction, sperm, sex ratio</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td>X</td><td>Translational and applied research</td></tr>
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<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>X</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">What are we investigating?

This project investigates the molecular processes required to form functional sperm, and how individual sperm differ in terms of fertilising ability. In particular, we are interested in genes on the X and Y chromosome that selectively affect the function of X- and Ybearing sperm and therefore affect the proportion of male and female offspring that are born — i.e. the offspring sex ratio.

We know that in mice, offspring sex ratio is affected by two genes, Six and Sly which are engaged in an “arms race” between the X and Y chromosomes. Sex ratio is controlled by the balance between Six and Sly copy number. Mice that have the same number of copies of each gene have a 50:50 sex ratio, while mice that have excess copies of Six have more female offspring and mice that have excess copies of Sly have more male offspring.

We want to find out how these genes cause their effects, and whether we can use this to regulate the sex ratio in farm animal species. In many farm species such as dairy cattle, female offspring are more valuable than males, while in other species the opposite situation holds. Currently, sex ratio balancing in farming is achieved by selective culling, which is</td></tr>
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	ethically undesirable and economically wasteful. Our work is currently at a pre-application stage. Once we understand the principles that govern sex ratio in our mouse model system, further work will be required to translate the findings to other species. **What experiments will we do?** To investigate this, we need to look at three scientific unknowns: 1) How genes on the sex chromosomes are switched on and off during sperm production. 2) How gene products are shared between developing sperm cells (in order for there to be a difference between X and Y sperm, there must be some genes that are not shared). 3) The quality control processes that remove damaged / nonfunctional sperm. We will study this by looking at tissues from mice that have genetic alterations that affect these aspects of sperm production. We will use the finding to develop new methods of selecting sperm or embryos to allow control of the offspring sex ratio, for example by using transgenes to “label” the X- or Y-bearing sperm with a molecular flag that allows sperm to be sorted. Most of our studies can be performed by looking at post mortem tissues from male mice, however from time to time we will need to collect eggs from female mice in order to test the fertilising potential of sperm from male mice of interest.
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)?	An estimated 100,000 male dairy calves and 30-40 million male layer chicks are culled per year in the UK alone. Male pigs are routinely either slaughtered before puberty (leading to economic wastage) or castrated (an animal welfare issue) due to “boar taint”. All of this would be unnecessary if alternative more humane methods of offspring sex selection can be developed as a result of our work. The basic scientific questions addressed by our work cover fundamental areas of reproduction, and the processes that control sperm production and quality control. This will be valuable to the scientific community in multiple areas of research into fertility.

What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 4510 mice over the course of five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The project does not in general require any harmful procedures other than production and breeding of transgenic and/or natural mutant mice. The mice are unlikely to suffer any pain or distress as part of these experiments, as the only known function of the genes we are looking at is to alter the shape and fertilising ability of the sperm. The vast majority (>4000) of animals used will not be. subject to any procedures other than breeding and/or injections of hormones or other harmless tracer substances. In the production of transgenic mice, a small number of animals will need surgical treatment to implant embryos (up to 200 females) or to undergo vasectomy (up to 10 males). These are minor procedures which will be carried out under anaesthetic and which will involve no more than temporary suffering (comparable to similar procedures in human fertility treatment). If egg collection is required, this will involve hormone treatment of the females (up to 2000) to increase egg production prior to collection of the egg cells post mortem. This will involve an injection that produces no more than transient suffering. All animals used will be humanely culled, and tissues taken post mortem for analysis if required.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no way to make sperm in cell culture and so live animal work is required to investigate sperm function. For the work investigating breeding and fertility: this necessarily requires live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For fundamental work examining gene function, wherever possible we will do experiments in cell culture. For the sex ratio testing, we have calculated the minimum number of animals required to achieve statistical significance. Where possible we will reduce the number of live animals used by carrying out experiments on cultured embryos (i.e. before the nervous system has developed so there is no possibility of animals suffering).

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 are using mice because the genes we are looking at, and the cellular processes involved, are better understood in mice than in other species. The project does not require any harmful procedures other than breeding. The mice are unlikely to suffer any pain or distress as part of these experiments, as the only known function of the genes we are looking at is to alter the shape and fertilising ability of the sperm. We have designed our “tagging” transgenes to carry fluorescent markers, meaning that the animals can be genotyped non-invasively without requiring biopsy.

Project 4	**Understanding and treating pregnancy related disorders**	
Key Words (max. 5 words)	Pregnancy, preterm labour, parturition, uterus, cervix	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the project is to understand how normal and abnormal labour starts, and how it can be treated or prevented.	
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)?	Ultimately we hope to be able to identify new treatments or tests for preterm labour OR for induction of labour. Around 50,000 babies are born preterm in the UK each year – these babies are at increased risk of premature death and of disabilities in later life. Induction of labour is an important strategy to reduce death of babies around the time of delivery: at the moment the drugs we have available for this have side effect, so we hope to be able to develop an alternative.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use around 3000 mice over the next five years.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	Many of the studies are involve trying to understand and prevent preterm labour. In order to achieve these aims, we stimulate preterm labour in some mice. Hence the adverse effects for the mothers and the	

level of severity? What will happen to the animals at the end?	pups include prematurity, which can be fatal. Some procedures used will result in pain (e.g. at injection site), however this will be minimised at all times by the use of painkillers, anaesthetics and close monitoring. The majority of our experiments are of low severity. The adverse effects of pro-inflammatory agents are sometimes of moderate severity, however these effects will be minimised at all times as described above.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are ONLY used when human work is not possible, and when work on cells in the laboratory is unsuitable, both because of the nature of the question being asked. An example is the testing of new agents for preterm birth prevention – such work cannot ethically be done in humans without showing first that they are effective in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our approach is to perform statistical analysis before we start the studies, to determine the minimum number of animals we need to use. We base these studies on our previous work.
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 minimal species required to address our research questions, and its biology is sufficiently similar to the human for it to give us helpful information. We have developed a major refinement of our preterm labour model, whereby we use ultrasound to guide the injection, hence avoiding having to do an operation on the animal. We monitor all animals carefully throughout the entire duration of the experiments. Where more refined approaches become available during the studies, we will adopt them.

Project 5	**Vitamin D and immune function**
Key Words	Vitamin D, Colon, pregnancy, placenta, inflammation
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Vitamin D-deficiency is a common problem across the globe, and may have health consequences that extend far beyond the classical effects of vitamin D on the skeleton. In particular vitamin D appears to have potent effects on the immune system, and vitamin D-deficiency has been proposed as a factor in some infectious and inflammatory diseases. Low vitamin D status appears to be particularly common in pregnant women, where it has been linked to adverse events in pregnancy such as pre-eclampsia. The underlying mechanisms for this are unclear but because the colon, spleen and placenta contain all the machinery required for activation and function of vitamin D, they are clearly key sites for vitamin D function. The aim of the current project is to determine how vitamin D contributes to normal immune function and then explore how this is disrupted under conditions of vitamin D-deficiency or loss of the vitamin D system in target tissues.

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)?

Although it has been established that many people are at risk of vitamin D-deficiency, our basic lack of understanding of what this means for human health is unclear. Vitamin D supplements are offered to many people, notably pregnant women, but the levels used in these supplements are very low and compliancy is poor. Studies of mouse models of vitamin D status and function may help to change this situation.

What types and approximate numbers of animals do you expect to use and over what period of time?

2150 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 levels of severity? What will happen to the animals at the end?

Protocols involve two strategies. In the first of these we will take normal wild type mice and place them on diets that have modifications in their vitamin D content (Sufficient/Deficient/Supplementary). Only the deficient and supplementary diets are listed as protocols because they are different from the normal diets, and there is a small risk of hypo- and hypercalcemia respectively. Some of these mice will also receive a DSS immune challenge, which is known to cause symptoms similar to colitis in humans. However, mice will only be exposed to this for 7 days, after which they will be allowed to recover. Some pregnant mice will receive another type of immune challenge in the form of a single intraperitoneal (IP) injection of an immune stimulator (LPS) during pregnancy. The dose of LPS will be low so as to have little effect on the mother. However, to monitor possible combined dietary/LPS effects we will maintain close watch of pregnant mice. Preliminary studies have shown no adverse effects with vitamin D-deficient diets and IP LPS. In all cases male mates in the study will receive no LPS. Similar studies will also be carried out using mice genetically altered to lack key genes in the vitamin D system. Most of these studies will use heterozygous mice (with one functional copy of the gene in question). When Heterozygous females are mated with heterozygous males, approx. 25% of the resulting foetuses and placentas lose both copies of the gene. We do not anticipate any additional adverse effects of the IP LPS in this part of the study as mice are normal and on a normal diet. Some studies will involve female mice who are homozygous knockouts for vitamin D genes (no copies of the gene in question). Two of the homozygous gene knockout mice (Vdr and Cyp27b1) are known to develop symptoms of hypocalcaemia. Whilst this occurs over longer age ranges than those to be used in the current protocols, we will nevertheless maintain these mice on an established ‘rescue’ diet which has extra calcium

Application of the 3Rs

Replacement

Studies of pregnancy *in vivo* are notoriously difficult to carry out. The precious nature of human pregnancies means that the sort of work we are planning is impossible. Mice are not truly a good alternative model for human pregnancy, but they do show similar pregnancy immune responses to humans. However, the fact that we are able to manipulate the vitamin D system in a highly reproducible fashion in these mice means that we can be more confident of successfully achieving the targets set out in the protocols. In addition, the studies we are planning for non-pregnant mice (e.g. DSS immune challenge) are based on previously reported studies that have allowed us to optimize treatment levels. As such, we are confident that successful mouse studies will allow us to return to human studies with greater assurance.

Reduction

As detailed in the project details, we have planned breeding experiments to obtain the maximum information from each mouse. This is particularly true during pregnancy where collection of multiple placenta tissues from one pregnancy means

that we can generate large amounts of new information from a very small number of pregnancies. In addition, the work we are proposing is based on preliminary studies in which we have carried out key analyses to be used in the current project. We can therefore obtain accurate data from smaller numbers of pregnancies.

Refinement

Mice have well a well-characterized vitamin D system, and we are therefore confident of obtaining meaningful data from the models we have proposed. In addition, the parameters we will measure (placental and immune health) have been well studied in mice. This allows us to use the least stressful approaches to studying mouse responses to vitamin D during pregnancy. We have also chosen challenges such as the single IP injection that have minimal stress. The genetically manipulated mice we have chosen can for the most part be used as heterozygous mice that have no health issues, with only the resulting foetuses showing complete gene knockout. Finally we have based dietary manipulation of vitamin D on previous studies where we have shown no severe effects of vitamin D-deficiency alone over the short periods of time outlined in this proposal. Based on these factors we have listed protocols as mild or moderate severity. However, the nature of the studies (pregnancy) means that we will carefully monitor all mice through pregnancy and specifically in the 24 hours following immune challenge.

Project 6	**Precursor cell therapies for kidney diseases**	
Key Words (max. 5 words)	Kidney Cell Mouse Therapy Scanning	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We will assess the potential therapeutic effects of precursor cells injected into mice with experimental kidney diseases. Our current studies show that: a. we have moise models which mimic human kidney disease; b. we have precursor cells ready for testing as new biological therapies for kidney disease; c. we have scans with which to monitor kidney disease and administered cells in living animals. In this project, we will bring these lines of work together to determine both where cells go after they have been administered to mice with experimental kidney disease, and whether administration of such cells prevent kidney damage. These studies will pave the way for the human trails evaluating human kidney precursor cell therapy.	
What are the potential benefits likely to derive from this project (how science could be advanced or	In the UK, 57,000 people have such severe kidney disease that they require long term dialysis or kidney transplantation. Unfortunately, being on long term dialysis confers a high risk of death, exceeding that	

humans or animals could benefit from the project)?	found in people who have certain cancers, and there are insufficient numbers of donors available to rescue all dialysis patients by kidney transplantation. Currently available drugs are only partially effective at treating kidney diseases. So, there is an urgent need to define new treatments to prevent people with diseased kidneys reaching a stage when they need dialysis. The current project, using mice with experimental kidney disease, is a step towards this end. Moreover, by using scanning, we will be able to track the fates of administered cells inside living animals, and these techniques may later be transferrable to humans (and also non-human animals) suffering from kidney diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. 650 over five years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The project has an overall 'Moderate' severity level. Mice with kidney injury will have limited and transient weight loss and behaviour changes (e.g. poor grooming). Similar transient changes may occur after surgery to block the tube called the ureter. These effects should not last longer than a week. At the end of each experiment the mouse will be killed by a humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We aim to determine whether the administration of precursor cells might be considered as a realistic therapeutic strategy for people with kidney disease. We, and others, have shown that such precursor cells can form kidney cells in culture. These experiments provide fascinating biological insights and are encouraging first steps towards therapies. However, neither normal kidney function nor the complex tissue changes of kidney disease, can be reproduced in cell culture. So, given the need for realistic preclinical models, there is currently no alternative to determining whether kidney precursor cells can treat kidney disease unless we use live animals.

	Moreover, the tracking of administered cells in whole animals is needed to gain insights into the mechanism of any beneficial effects they may be found to have.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of scans to monitor both the structure and function of kidneys, and also to locate labelled precursor cells which have been injected into mice with kidney disease, is a key feature of this project. While these techniques do require general anaesthesia, and sometimes also injection of chemicals, they are 'minimally invasive' and so can be used recurrently on a single mouse to monitor disease progression, obtaining a large amount and variety of data. This will reduce the total number of mice used in the study. The scanning results will be compared with kidney fibrosis found at autopsy. Such tissue analyses have previously been a 'gold standard' of mouse kidney experiments. In our preliminary experiments, we have quantified the proportion of the kidney occupied by scarred tissue. This allows us to calculate the minimum numbers of mice we would need to use to demonstrate a statistically significant and biologically important therapeutic effect.
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.	At present, the mouse represents the best species with which to test the efficacy of precursor cell therapies. Progressive kidney disease which mimics human kidney disease has not yet been modelled in lower species such as fish and frogs. The mouse has a kidney of similar structure and anatomical complexity to humans. Moreover, mouse disease models can reproduce the anatomic and functional changes that occur in human kidney disease. Mice will be closely monitored (several times a day soon after the induction of kidney damage, and then daily during the 'chronic' phase of kidney disease. Particular attention will be paid to their weights and behaviour. Should these parameters deviate markedly from normal, mice will be humanely killed. Rather than collect urine using metabolic cages (which is stressful for mice), we will use spontaneously voided 'spot urine' samples.

Project 7	**Improving organ function in kidney transplantation**	
Key Words (max. 5 words)	Renal, transplant, therapy, regenerative	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	1. Improve the rat model to better mimic the human transplant situation 2. Investigate the mechanisms whereby regenerative therapies help kidney injury. 3. Determine any other potentially harmful effects of the cells on the body	
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 number of patients developing chronic kidney disease and progressive renal failure is escalating on a yearly basis, Current Renal association figures estimate as many as 60000 patients require maintenance renal replacement therapy and this number increases yearly resulting in exponentially increasing costs to the National Health Service. The optimum treatment for patient survival and quality of life is renal transplantation but this excellent treatment has the inherent limitation of organ supply. The average lifespan of a transplanted kidney is ten to fifteen years. Most kidneys eventually fail due to a combination of injurious processes both following the transplant but also associated with the events at the actual time of implantation. Current clinical strategies essentially have no proven intervention, which reliably protects transplanted	

	kidneys against this transplant-associated injury. However, there is now evidence that fat derived regenerative cells administered to rats, with this transplanted associated injury, have significantly decreased mortality and increase renal function. Therefore the potential benefits: To learn how the therapies found to improve kidney function work and refine/improve their effect. To identify any risk of toxicity or unexpected effects these therapies may have. To learn where in the body, therapeutic cells injected into kidneys end up. These findings could make kidneys currently discarded become useable for transplantation. More patients would then receive the best possible treatment — a kidney transplant. Currently transplanted kidneys could work better and for longer from the knowledge gained
What species and approximate numbers of animals do you expect to use over what period of time?	Species: Rat Approximate number: 800 Time period: 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The main potential adverse effects that the rats could face are the risks associated with surgery. It is unlikely that any rat will suffer from the intervention or change in physiology- they will be susceptible to the known side effects of any - operation such bleeding, infection, risk of anaesthetic and postoperative pain. Systems are in place to reduce the chance of these adverse effects and to recognize and treat any of these complications quickly. There are well-defined end points for each protocol and sacrifice of the animal will only be via home office approved methods. If any adverse effects are noted prior to the end of a procedure or if there is a breach in the severity limit, the animal will be brought to the attention of the NVS or will be euthanised.
Application of the 3Rs	
1. Replacement	The proposed work builds on a background of extensive animal work already published from institutes

State why you need to use animals and why you cannot use non-animal alternatives	around the world. Renal function is complex and any intervention to treat kidney injury would require investigations of the kidney as a whole, with an intact circulation. We also want to make sure that cell therapy does not affect other organ systems. Only work on animals with vascular and urinary tract intervention can facilitate the study of this area. The validity of this model is confirmed by the fact that a variety of drug agents established in clinical transplantation have been developed via this model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experimental protocols and the method of statistical analysis have been discussed with supporting statistical experts to confirm appropriate tower and number required. All experiments will be conducted in line with good laboratory practice with a specified statement of objectives, detailed protocol, end points, and plan of statistical analysis/level of significance sought.
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 protocols entailed in this project represent the most refined approach possible to the clinical problem studied. Wherever possible in all protocols, invasive procedures are carried out under terminal anaesthesia to minimise animal suffering. In all protocols incorporating anaesthesia and surgical procedures, animals will receive appropriate analgesia, warmth and supplementary food and water. All animals will he observed for any signs of significant distress and where unexpected suffering is suspected, the animal will be humanely killed. One of the protocols has an overall severity of severe. This rating reflects the technical complexity of microsurgery in rodents and its associated learning curve. When technically successful, protocols will be no more than moderate in severity and that will be the modal level for animals treated in these protocols however given the complexity of the procedure, it is inevitable that a small proportion of animals will — even with the greatest possible operator skill and experience — undergo some suffering as a result of haemorrhage or blockage of a major vessel. In every case, an assessment of the likelihood of such a complication will be made before recovery and any animals at unacceptable risk will be humanely killed.

	Operating personal licensees undergo an approved animal microsurgery training course and commence a period of training initially on humanely killed animals and subsequently on a number of live animals which will be humanely killed before recovery (n approximately =10) under supervision. Once a success rate of >80% is expected the operator will commence independent practice. We work closely with an expert in the rat kidney model, to ensure minimal animal suffering with regard to the altered functioning of the kidney.

Project 8	**Rodent models to understand kidney disease**	
Key Words (max. 5 words)	Rodent models, kidney disease	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To discover the important cellular signalling pathways necessary for kidney function and how these change in kidney disease situations.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work will increase our understanding of how the kidney works. It will reveal important cellular signalling pathways in the kidney and also identify key molecules that can be targeted by drugs to prevent or cure common kidney diseases experienced by our patients.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats. Approximately 6000 animals 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	Mice will be either bred to knock-out or over-express proteins in the cells of the filtering unit of the kidney. This is generally painless for the mice and doesn't cause them any harm. We are able to detect if changing these proteins are affecting the kidney of	

happen to the animals at the end?	the animal by looking for protein in their urine. We will also give some animals kidney diseases that are similar to those experienced by our patients including diabetes and haemolytic uraemic syndrome. This is to try and understand why these kidney diseases occur and to find new treatments for them. The majority of our procedures are painless but for those that are we will give the animals regular pain-killers. In a small number of animals we will perform kidney operations on them using general anaesthetic. We will ensure they experience minimal pain and suffering by giving them regular pain-killers while they are recovering. If mice get severe kidney disease this may cause them to die. However it is normally due to changes in salts in the body and is generally not painful. The majority of work will be of mild severity. We have some models that are of severe severity. These models are copying severe kidney diseases in our patients. Again when we study these we will ensure the mice have good levels of pain relief in order to minimise any suffering. At the end of our procedures the mice and rats will be put to sleep.
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 as this is the only way we can study pathways in a kidney which is acting in the same way our patients and is being perfused with blood.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the minimal amount of animals by also using other techniques to study kidney cells including studying isolated kidney cells in dishes (both rodent and human). We will also set up breeding schemes to minimise the amount of mice that we need for our studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	We are using the simplest mammalian model that reproduces that in man, which are mice and rats. These species have kidneys that are similar to humans in respect to their blood flow and structure. We will ensure all measures are taken to minimise any suffering to the rodents we study including

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	enriching their cage environment, monitoring them regularly to ensure they are not suffering and using appropriate measures to reduce any adverse effects they may experience. Some of our models are severe as they are studying severe human kidney conditions. For these we will ensure the animals experience as little harm as possible by identifying sick animals early through examination and either putting them to sleep or giving them appropriate treatments or painkillers as necessary.

Project 9	**The effect of aging on tissue injury and repair**	
Key Words (max. 5 words)	Kidney, acute injury, chronic fibrosis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	With increasing age, humans become more susceptible to acute kidney injury (AKI) and scarring and exhibit a reduced ability to repair the kidney leading to a worsened long-term outcome of disease. The mechanisms underlying the adverse impact of ageing upon disease of the kidney as well as other organs remain poorly understood. This project aims to identify factors underlying the poorer response of the kidney to acute insults with increasing age, by studying this in mice.	
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 aim of this project is to identify processes that are altered in older animals which account for their worsened outcome during and after kidney injury. The pathways underpinning these processes could then be targeted and modulated with drugs, to reduce the risk of acute kidney injury, prevent excessive scarring and improve the ability of the kidney to recover fully from insults. Such interventions would be predicted to have a beneficial effect upon both short-term and long-term illness and deaths in man.	

What species and approximate numbers of animals do you expect to use over what period of time?	All experiments will be performed in mice. I estimate around 4250 mice will be used over the five year course 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 end?	The main adverse events anticipated would relate to signs of acute kidney injury and these would likely be moderate in severity, and result in a mouse losing weight, and showing signs of illness such as reduced activity and hunched posture. The risk of post-operative wound infections will be minimized by the use of full aseptic technique. All animals will be closely monitored and adequate analgesia administered. Animals will be humanely killed at the end of experiments to allow collection of tissue samples for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Kidney failure is a complex and poorly understood disease encompassing both systemic effects and injury to multiple cell types. There is no in vitro or computer based simulation that can adequately replace carefully controlled in vivo experimentation at present. We will use carefully selected in vitro assays of renal cell behaviour to add value to the animal studies performed, and to minimise the need for in vivo experimentation as much as possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have extensive experience in the chosen disease models. Statistical analysis of our previous published studies indicate that experimental groups of 10 animals are necessary to generate robust data. When new experiments are undertaken we will undertake additional power calculations whenever possible to calculate the most appropriate 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.	Mice will be used in light of the ready availability of genetically modified mice available for study of disease pathways and processes. We have selected models of acute kidney injury and progressive kidney fibrosis that equate to human disease. We will use a short-term kidney blood flow restriction model (IRI) and a urine flow restriction model (UUO) both of which are good models for acute kidney injury in people. We are familiar with these models and have refined them to use the minimum required kidney damage by reducing the time of blood flow restriction, limiting the degree of suffering experienced by the animals. We will always seek veterinary input in the event of any concerns about the condition of mice under this protocol, to ensure that signs of distress are not missed. Pain killers are always administered before and after surgery to limit discomfort experienced by the mice. Humane endpoints will always be designed for all experiments.

Project 10	**Endometrial function and associated disorders**	
Key Words (max. 5 words)	Uterus, steroid, inflammation, repair, endometriosis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	These studies will explore the mechanisms that regulate uterine (womb) function. The major focus of our investigation will be on the impact of sex steroids such as androgens and oestrogens on the endometrium. This complex multicellular tissue is located in the centre of the uterus. During a fertile cycle the embryo attaches to the endometrium and develops a placenta that nurtures the development of the fetus throughout pregnancy. If there is no pregnancy in women the inner 1/3 of the endometrium breaks down and is shed an event experienced as a 'period'. A secondary objective of these studies is study fundamental hormone regulated processes in the endometrium including inflammation, formation of new blood vessels (angiogenesis) and recruitment of and differentiation of progenitor (stem) cells.	
What are the potential benefits likely to derive from this project (how science could be advanced or	These studies will provide important, insights into the regulation of the endometrium a tissue that plays a fundamental role in establishment of pregnancy in both women and all other mammals. Endometrial disorders	

humans or animals could benefit from the project)?	including endometriosis, heavy menstrual bleeding and infertility affect many millions of women — symptoms can be debilitating resulting in time off work and a negative impact on families. The fundamental insights gained from these studies will inform development of new medical therapies for endometriosis and other endometrial disorders such as heavy periods.
What species and approximate numbers of animals do you expect to use over what period of time?	Studies will be conducted in mice as they have a uterus with endometrial tissue that contains the same arrangement of cells as in women. We expect to use no more than 12,000 mice over 5 years: numbers bred will be kept to a minimum and we will collaborate with others to maximise use of tissues.
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?	Mice will be houses in groups with unrestricted access to food and water. We will only be using genetically modified animals when they can provide unique insights into mechanisms regulating endometrial function and we do not envisage any of them having health problems as a result of their genetic change. We have established robust protocols to model both menstruation (periods) and endometriosis in mice in such a way that they reproduce these events in women. Both models involve minor surgery under anaesthetic and any discomfort is mininiised using post surgical analgesics. We are not anticipating adverse impacts of genetic modification on the general health of the mice.genetically modified mice are mild and non-life threatening. Animals will be humanely killed or killed under anaesthetic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We make extensive use of human cells and tissues but even with improvements in our culture systems including use of 3D and co-cultures we cannot recapitulate the complex architecture of the uterus in vitro. Mammals such as mice have a uterus with an endometrium that is responds to sex steroid hormones as in women. Species such as flies and worms do not have a uterus or endometrium.
2. Reduction	We will use the minimum number of mice to comply with our power calculations for group size ensuring

Explain how you will assure the use of minimum numbers of animals	results achieve significant endpoints. In the case of genetically modified mice as we will only be using females we have developed collaborators with others who can use male mice for their studies so that no mice need to be culled unnecessarily.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the smallest mammalian species in which we can study the function of the endometrium; access to inbred strains (reduces variation in response) as well as mice with genetic modifications that change expression of genes implicated in reproductive health of women make them the animal of choice. The genome of mice has been fully annotated and datasets exist both from our own and other studies world wide which we can use for comparison when considering novel data. We have developed and refined the mouse models of menstruation and endometriosis and will study the mice using non-invasive tests such as imaging and behavioural testing as this enables us to learn more about their response to drugs including hormones. At the end of the experiment we will recover a comprehensive set of samples from each mouse so that we can reduce the number of animals used in each study. Data will be shared with others working in the same field to avoid duplication.

Project 11	**Optimising ovarian tissue transplantation by improving vascularisation**	
Key Words (max. 5 words)	Fertility preservation, xenografting, nude mouse, human ovarian tissue, transplantation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The results of this study will show us, which of the three methods, alone or in combination, most effectively reduces the damage to the ovarian tissue during transplantation and will be an important step toward the goal of providing an efficient way to improve the quality of life for women treated for cancer by restoring their ability to conceive.	
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 a focus group conducted with patients of reproductive age who were undergoing cancer treatment found that a means of preserving their fertility successfully would be of great comfort to them. A cancer diagnosis alone is extremely daunting and it is further compounded by the adverse side effects of receiving the life saving treatment. The preliminary phases of this study have lead to implementing a tissue-freezing program.; As such the primary outcome of this work would allow for these tissues to be transplanted back to the women currently taking part in	

	the tissue freezing program, thus giving these women, who have been recently diagnosed with cancer, a chance of conceiving a child once they are in remission.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using 250 nude mice in total for this project, over the course of the five-year duration of the licence.
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?	Each animal used in this project will have a small operation under general anaesthetic, where two small pieces of human ovarian tissue will be transplanted onto the inner lining of the abdomen. We do not anticipate anything more than some minor bleeding from the skin and abdominal wall, which will be stopped immediately during the operation. The animals may experience some post-operative pain, which will be controlled by the use of pain-killing agents. After 5 months, the animals will receive a total of six injections of human hormones on alternate days. The injection sites will be alternated, and we expect that these injections will only cause momentary needle-stick pain. The likely severity level of these interventions is moderate. All animals will be killed humanely at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is difficult to use a non-animal model as without in-vivo hormonal stimulation there is little success in maturing follicles.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have performed preliminary studies in vitro to refine the best po3sible transplantation method for the animal studies to minimise the number of animals used in the project. We have involved a statistician in our experimental design to ensure that the maximum amount of useful results can be obtained from using the minimum number of animals throughout the project.
3. Refinement	The nude mouse provides a very good mammalian environment for the growth and maintenance of human

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.	ovarian tissue, and does not reject it as foreign due to having an incomplete immune system. This makes them the most appropriate animal to use in this project. Experienced practitioners will perform the surgical procedures with the animals under general anaesthetic, and the transplanted tissue will be kept small to minimise the effects on the animal. Pain-killing agents will be used to ensure the animals are comfortable post-operatively.

Project 12	**Mechanism of bladder cancer and translation**	
Key Words (max. 5 words)	Cre-LoxP, carcinogen, FGFR3, CXCR2, cell free DNA	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Bladder cancer is common and significant proportion of patients die from this disease. The effective therapy is lacking and development of new drugs is one of the main priorities. The treatment of bladder cancer is known to be expensive, mostly due to the costly therapies and necessity of repeated monitoring of the recurrent disease. Overall aim of this research is to identify new or improved therapeutic drugs and to develop diagnostic tools that have a potential to be applied for the benefit of patients as soon as possible. The scientific goals are to obtain understanding and knowledge of the mechanism of bladder cancer progression caused by changes in growth factor/cytokine signalling, immune responses and tumour microenvironment, and whether potential therapies against these factors can suppress bladder cancer. We will also test whether tumour DNA can be detected in blood and urine of mouse models with bladder tumours.	

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)?	Therapies used in other cancer types are often applied in bladder cancer with an assumption that cancer cells behave in a similar way. Although more genetic knowledge in bladder cancer has become available, it remains unclear whether these gene alterations actually play a functional role. The immune responses and its changes that occur closely surrounding the tumour was shown to play an important role in tumour progression in many cancer types. However in bladder cancer, more evidence is needed. Knowledge and understanding of specific factors that influence bladder cancer progression will enable new and improved therapeutic approaches to bladder cancer specifically, and they are likely to be used in patients sooner. Successful detection of tumour DNA in blood and urine of bladder cancer models will serve as a foundation of further studies that eventually reduce the overall number of animals used for research and to reduce suffering in each animal while maximizing the information that can be obtained. 2900 mice will be used
What species and approximate numbers of animals do you expect to use over what period of time?	2900 mice 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?	Half of these animals will be used for breeding of progenies to generate the model with intended gene modifications. Most of these animals are not expected to suffer from adverse effects of bladder cancer or animal handling procedures. The other half may develop moderate levels of severity caused by formation of bladder cancer or may go through procedures such as repeated administration of therapeutic drugs. Severity of tumours will be examined by non-invasive imaging in vivo upon therapeutic treatment and humanely killed at the intended time point before tumour causes further suffering. Tumours will be examined in the laboratory after animal are humanely killed. More than half of the animals will be humanely hilled earlier than such suffering is caused in order to study the process of

	tumour development.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will use genetically modified mice with mutations highly associated with bladder cancers in humans. No other models are currently available to meet the purpose of the proposed study. Bladder cancers originate in the inner wall of the bladder that forms a unique structure made of highly specialized cells. Difficulties in modelling tumours in the bladder are well known. However, our group and others showed that mice could model features similar to bladder cancers in humans. Bladder tumours are one of the cancer types that develop late in life, where mutations in many genes are likely to contribute to the tumour initiation and progression. Cancer cell lines, even though they may be obtained from patients, often lack the essential characteristics of bladder cancer. Profiles of gene mutations in our mouse models are likely to much better reflect bladder pathogenesis than in cell culture. Due to the technical availabilities and cost, use the three- dimensional cell culture system is currently unrealistic.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that the minimum number of animals will be used to generate scientifically meaningful and clinically useful results. Statistical methods will be appropriately used to estimate the number of animals needed. The results are compared to other studies and experiences of our group and others. The majority of mouse strains we intend to use are on a uniform genetic background, therefore variations in phenotype or problem in breeding are minimized. The use of in vivo imaging to assess tumour burden, including ultrasound, allows us to monitor the same animal in the course of tumourigenesis and thus reduces the requirement of mice at a number of different time points. Whenever possible, mouse lines are shared with other projects to reduce the number of mice bred.
3. Refinement Explain the choice of species and why the animal model(s)	We will ensure that all animals receive the highest standard of care, and where appropriate, social, environmental and behavioural enrichment will be provided. Close monitoring on tumour development will

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.	ensure animal suffering is kept to a minimum. The bladder tissues are specifically targeted and the off-target adverse effects in the animal is avoided as much as possible. We have gained extensive experiences of bladder cancer mouse models over the last 5 years. We have hands-on knowledge of the clinical signs that the specific models may develop. This allows us to detect and care for animals exhibiting mild or moderate clinical signs at an earlier stage. The use of in vivo imaging allows us to detect internal tumours at much earlier stages of tumourigenesis. In this project, we also intend to develop non-invasive method of tumour detection and genotyping by use of cell-free DNA. If successful, this will minimize the animal suffering while enhancing the information that can be retrieved from tumours and animals.

Project 13	Optimisation of drug efficacy and safety in bladder cancer
Key Words	Cancer, Chemotherapy, Toxicity
Expected duration of the project	3 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

In many forms of cancer, including bladder cancer, the effectiveness of chemotherapy drugs can gradually decline as tumours develop resistance. One of the main causes of this resistance is the enhanced activity of cell defence pathways that allow cancer cells to inactivate and remove chemotherapy drugs, thus limiting their effectiveness. Experiments involving cultured cancer cells have shown that inhibition of these enhanced cell defence processes can overcome resistance to some chemotherapy drugs, restoring their effectiveness. However, before such a strategy can be used in patients, there is a need to investigate the benefits and risks of modulating cell defence in animals. Specifically, given that chemotherapy drugs are known to cause side effects via toxicity to normal cells, it will be important to determine if manipulation of cell defence processes increases the risk/severity of side effects associated with chemotherapy drugs, or whether it is possible to overcome resistance to chemotherapy without worsening side effects.

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)?

If we can provide evidence that modulation of cell defence processes can enhance the sensitivity of bladder cancer cells to chemotherapeutic agents without worsening adverse effects in other organs, the knowledge gained from this project will inform the design of early-phase clinical trials designed to test the value of this novel therapeutic strategy in cancer patients. This could ultimately improve our ability to treat bladder (and other forms of) cancer through the personalisation of therapy.

Alternatively, if our studies reveal that modulation of cell defence processes does enhance the unwanted side effects of chemotherapeutic drugs, this will signify the likely inappropriateness of this approach in clinical practice and thus avoid the unnecessary exposure of patients to a harmful treatment regime.

What types and approximate numbers of animals do you expect to use and over what period of time?

We expect to use up to 750 mice and 250 rats in studies over a 3 year period.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Most studies will involve the stimulation of tumour growth, in order to assess the effectiveness of chemotherapeutic drugs (in combination with cell defence modulating agents) on tumour development. These studies would fall into the moderate severity category. Some animals will be subjected to moderate surgical procedures, conducted under anaesthesia and with appropriate post-surgery pain relief, to allow implantation of cancer cells/tissue. We will closely monitor tumour size and stop any studies in which this exceeds a maximum value. Substances will be administered, usually by injection, at less than or equal to the maximum tolerated dose. Animals will be humanely culled if they display signs of significant ill health in response to tumour formation and/or substance administration. At the end of all procedures, animals will be humanely culled and tissues will be recovered for further analysis.

Application of the 3Rs

Replacement

Standard cell/tissue culture techniques are informative (and will be used whenever possible/appropriate) but unable to mimic the complex environment that underpins tumour development in a living body. Also, to address the main aim of this project, we need to investigate chemotherapeutic drug efficacy (towards cancer cells/tumours) and toxic side effects (towards normal cells/organs) simultaneously in a single model. Such studies cannot be performed with existing cell/tissue culture techniques.

Reduction

Whenever possible, we will use cell/tissue culture techniques and human tissue biopsies to address our research questions. When necessary, we will ensure the most efficient use of animals by using statistical power analysis to determine group sizes. We estimate that 12 and 30 animals per experimental group will be required for typical studies involving grafting of cancer cells/tissue and chemical induction of tumour formation, respectively. Where possible, animal usage will be minimised by exploiting our ability to monitor tumour growth and response to treatment using

minimally-invasive imaging techniques. These techniques allow repeated measurements to be taken over time in the same animal, limiting the need for large group sizes.

Refinement

Our studies will involve the use of mice and rats, as several useful experimental models of bladder cancer have been established in these species, and because they allow us to evaluate chemotherapeutic drug effectiveness and adverse effects simultaneously. Data from previous and future experiments will be used to review the minimum doses of substances required to exert biological effects, in order to avoid doses that induce significant toxicity. We will use humane endpoints to determine when studies must be stopped to minimise animal distress. We will keep abreast of developments in animal-free testing of chemotherapy drug effectiveness and side effects and, if possible, incorporate such advances into our overall programme of work.

Project 14	PKCα and vascular calcification in kidney dysfunction
Key Words	PKCα, Vascular calcification, Chronic kidney disease
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Bone-like calcium deposits are found in the walls of blood vessels of over 80% of patients with chronic kidney disease (CKD), diabetes and atherosclerosis. These deposits contribute to a reduction in the quality of life and premature death. Despite the serious nature of this condition there is no effective treatment available to patients.

Extensive studies performed using isolated cells in culture have shown that an enzyme, protein kinase Cα (PKCα), is a regulator of calcium deposition in blood vessels (vascular calcification). Before we can transfer these basic observations into a potential therapy, we need to confirm that PKCα regulates vascular calcification in the more complex whole body setting.

The aim of this project is to establish whether PKCα regulates the vascular calcification that occurs as a result of CKD. If PKCα is shown to be a regulator of vascular calcification we aim to develop therapeutic interventions which target PKCα with a view to slowing down or preventing vascular calcification.

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 fatal consequences of vascular calcification for the large and growing number of patients with CKD, and the potential of this study to identify a novel drug target for this pathology, make this project necessary and worthwhile. In the short-term, we will determine whether PKCα plays a role in the development of vascular calcification

that occurs as a result of renal dysfunction. This information would be a major advancement of our current knowledge of the biological regulation of vascular calcification in chronic kidney disease. In the longer term, the knowledge gained from this project could justify larger scale animal trials of drugs that modulate PKCα, with the aim of moving onto human clinical trials in the future.

What types and approximate numbers of animals do you expect to use and over what period of time?

We have requested authority to perform experiments under this licence for the standard 5 year period. We will use mice for the planned experiments. Some of these mice will be normal animals that will be used as controls against which to compare differences. The remaining mice will have been genetically modified such that they are unable to make PKCα. We will study both male and female mice, unless it becomes apparent that one of the sexes is un-responsive to the experimental manipulation in which case we will discontinue using that sex. In order to set up a breeding colony to supply sufficient mice lacking PKCα for the experimental work we will require 700 mice. In order to determine how long we need to treat mice with a diet enriched with phosphate in order to induce vascular calcification, we will perform a pilot study using a total of 48 mice. Once we have established the most appropriate time scale, we will conduct the main experiment using both normal animals and mice lacking PKCα, potentially of both sexes. Based on our previous experience of this experimental approach we have calculated that we will need up to 17 mice per group to detect meaningful differences in the degree of calcification. We will also need a further 10-15 mice per group for follow up experiments designed to identify the underlying molecular mechanisms. These experiments will require up to 152 mice if we study both sexes. The total number of mice that we are likely to require for breeding = 700 and the total number of mice for experimental purposes = 200.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The severity limit of this project is moderate, because animals will undergo invasive surgery and then be allowed to recover. The surgery itself carries a small risk of acute renal failure (< 5% in our hands). Post-operative pain will be prevented through the use of analgesics. The combination of renal reduction surgery and a high phosphate diet is designed to induce accelerated vascular calcification which would eventually lead to heart attack or a stroke. However the time-frame of the experiment is such that mice will not experience these effects. Mice are expected to develop chronic kidney disease which is ultimately fatal. However once again this will not occur within the time-frame of the experiment. In order to monitor the health of the animals we will periodically take blood samples. Mice will experience transient discomfort from a needle prick required to collect blood. At the end of the

experiments all mice will be killed by a Schedule 1 method or under terminal anaesthesia.

Application of the 3Rs

Replacement

CKD leads to complex changes in physiological function, both within the diseased kidney and in the body as a whole. Many of these changes are not understood fully and so cannot be reproduced using cell culture or computer modelling. However it is precisely these systemic changes that result in vascular calcification. Consequently, it is necessary to study the changes that occur in blood vessels in the intact animal in order to understand what is happening as CKD progresses. This leaves us with no viable alternative to the use of animals for the proposed project.

Reduction

We have designed our experiments so that we are able to gain the most possible information from each individual animal. We have performed sample size calculations based on our own experience with the animal model and that of others using similar approaches so that we know how many mice will be required to give statistically meaningful results. We have built in a pilot study to establish whether both sexes need to be studied.

Refinement

We will use mice in this study because they share similar physiology with humans. They can also be manipulated genetically with ease, allowing us to study our target molecule of interest. We will use a model that involves reducing the mass of the kidneys and feeding a high phosphate diet in order to induce accelerated vascular calcification. The surgical approach itself is recognized as being a good model of human CKD. The addition of a high phosphate diet means that mice will develop the pathology of interest quickly so that they do not have to be kept for an extended period of time. We have built in a pilot study to determine how long we need to feed mice a high phosphate diet in order to produce a detectable change in vascular calcification so that the length of the experiment is optimised. Clearly defined humane endpoints have been built in to the project to ensure that animals do not suffer unnecessarily.

Project 15	Decellularised biomaterials for homologous use in urinary bladder auto-augmentation
Key Words	biomaterial, urinary tract, detrusorotomy, bladder
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

A number of congenital and acquired diseases of the urinary bladder culminate in end-stage disease characterised by small contracted bladders that are susceptible to recurrent infections and create high pressure systems that can cause irreversible damage to the kidneys.

Reconstructive surgery in end-stage disease aims to reduce pressures in order to prevent kidney damage. An option is the division of the main bladder muscle (detrusor) and scar tissue in order to make the inner lining of the bladder (mucosa) bulge, increasing bladder capacity and hence reducing bladder pressures; this procedure is known as auto-augmentation or detrusorotomy. Because the risk of perforation there have been some attempts to cover the mucosa with flaps from a variety of vascularised tissues within the patient.

Plan of work and impact of our studies: Our proposal is to replace the use of this surrogate tissue with a bladder grafts from other sources that have had all cellular material removed.

We have previously developed a procedure that enables the decellularisation of porcine bladders to generate a full thickness porcine acellular bladder matrix (PABM) which retains material properties of the bladder. We believe PABM will be useful on an autoaugmented bladder to provide support to the exposed mucosa. The properties of PABM make it highly attractive for use as a surgical patch material for use in the lower urinary tract.

Recently, we have made progress in converting the original laboratory bladder decellularisation protocol into a procedure more suitable for batch processing and scale up. We are now keen to apply this to test the concept of PABM patch detrusorotomy in a large animal series. In the longer term, a full thickness acellular bladder matrix of porcine derivation could solve a major unmet clinical need as a tissue-integrative patch material in urinary bladder auto-augmentation.

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 contracted bladders. It is likely that the material will be useful for both adult cases and also children undergoing their first operation. This will benefit the patient's short term and long term welfare by reducing the risk of bladder perforation and renal failure, ideally 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 types and approximate numbers of animals do you expect to use and over what period of time?

It is anticipated that the new scaffold could revolutionise surgical treatment of contracted bladders. It is likely that the material will be useful for both adult cases and also children undergoing their first operation. This will benefit the patient's short term and long term welfare by reducing the risk of bladder perforation and renal failure, ideally 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.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Possible side effects are transient discomfort, pain, wound infection, voiding difficulties, herniation of abdominal organs. At the end of the experiment, the animals will be humanely killed and tissues collected for histological examination.

Application of the 3Rs

Replacement

We have extensively studied the properties of PABM *in vitro*. To take this forward safely into routine surgical practice, we need to investigate the use of PABM in surgical animal model. This can only be performed in an animal equivalent to humans in respect of size, anatomy, morphology and function.

Reduction

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 successfully completed. We will start with 6 pigs, then followed by another 6. We will only use all 12 animals in total if first series is completed successfully.

Refinement

The operations will be performed by a Paediatric Urologist Surgeon who routinely performs similar operations in children and he will be assisted by a Clinical Research Fellow (fully surgically trained) and a Veterinary Surgeon. The pig has been selected as they are equivalent to humans in respect of size, anatomy, morphology and function.

Project 16	Modelling therapies for renal malformations
Key Words	Kidney, ureter, bladder, malformation, therapy
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

We will assess the feasibility and efficacy of new therapies to treat kidney and urinary tractmalformations. Our studies show that: a. we have animal models with kidney and urinary tract malformations and genetic defects similar to those found in patients. b. we understand the biological mechanisms why development is going wrong in these animals. c. we have potential new medicinal therapies, such as growth factors', to make the kidneys and urinary tracts grow normally. In this project, we will bring these lines of work together to treat animals with these therapies. These studies will pave the way for the human trails evaluating similar therapies.

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)?

In the UK, 1000 children have such severe kidney disease that they require long term dialysis or kidney transplantation. Half of them were born with abnormal kidneys and urinary tracts. Moreover, around 5,000 UK adults with severe renal failure were born with similar malformations. These numbers exclude individuals found on fetal screening to have kidney and urinary tract malformations who subsequently die after elective termination of pregnancy. Being on long term dialysis confers a high risk of death, exceeding that found in certain cancers, and there are insufficient numbers of donors available to rescue all dialysis patients by kidney transplantation. Moreover, treatments for these kidney patients costs £30-70,000/year and they comprise a great social burden on affected families. Currently, no treatments exist to prevent the malformations themselves. So, there is an urgent need to define new treatments for

these conditions. The current project, using animals with experimental disease, is a step towards this end.

What types and approximate numbers of animals do you expect to use and over what period of time?

(N.B. these numbers exclude embryonic forms) Mice 5000 over five years (of which approximately 1500 will be fetuses in the last third of gestation) Frogs (Xenopus) 2000 over five years (of which 1000 will be free-feeding tadpoles)

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The project has a ‘Moderate’ severity level. We will use breeding programmes so that, typically, clinically healthy parents (each carrying a mutant gene) are mated to produce litters containing animals with two mutant genes. The latter animals will have kidney and urinary tract malformations. Treatments (e.g. growth factors transferred by non harmful virus vectors) will be delivered to embryos via the mother, or directly into embryos, or into baby animals.In some experiments, we will study mouse embryos and fetuses, and frog embryos and larvae. In other experiments, when the malformations are anatomically mild or moderate, we will cautiously follow the progress of mice in the year after birth. Should signs of ill health become apparent, the animal will be killed by a humane method.

Application of the 3Rs

Replacement

We aim to determine whether therapies, for example using ‘growth factors’, can prevent or treat kidney and urinary tract malformations. We, and others, have shown that human stem cells can form ‘mini kidneys’ in culture. These experiments provide fascinating biological insights and we are using them as test beds for therapies. However, neither normal kidney function nor the complex tissue changes of kidney disease, can currently be reproduced in cell culture. So, given the need for realistic preclinical models, there is currently no alternative to using live animals Moreover, the administration of treatments to whole animals ensures that we can detect any (albeit unanticipated effects) on other organs.

Reduction

The use of (e.g. ultrasound and magnetic resonance imaging) scans to monitor both the structure of kidneys and the urinary tract is a feature of this project. While these techniques require general anaesthesia, they are ‘minimally invasive’ and so can be used recurrently on a single mouse to monitor disease, obtaining a large amount and variety of data. This will reduce the total number of mice used in the study. The scanning results will be compared with kidney and urinary tract at autopsy.

Healthy wild type and heterozygous control mice generated in Protocol 3 may be re-used for breeding in Protocols 1 and 2. This ensures that they are used to full effect and not killed as redundant mice.

Refinement

At present, the mouse represents the best species with which to test the efficacy of new kidney therapies. The mouse has a kidney of similar structure and anatomical complexity (e.g. with glomeruli and branching collecting ducts) to humans.

In many experiments, we will study embryonic and fetal animals that have malformations. Note that, in mice, the kidneys are not needed for life before birth because the placenta gets rid of fetal waste products.

In other experiments, will be closely monitored after birth. Particular attention will be paid to their weights and behaviour. Should these parameters deviate markedly from normal, mice will be humanely killed.

We will also study the frog called Xenopus, a lower organism. It has a very simple embryonic kidney, with a glomerulus and tubule similar to those found in mammals. Its embryonic kidney, however, lacks branching tubules that are often at fault in human kidney malformations. Moreover, the embryonic frog does not have a ureter or physiologically functional bladder. Despite the above limitations, the inclusion of frogs in the current study is highly desirable because large numbers of embryos can be quickly generated and it is simple to manipulate gene expression in these embryos. Thus, the information obtained from frog studies can complement and inform the mouse studies.

Project 17	The Causes and Treatment of rejection of kidney transplant
Key Words	alloimunity, inflammation, tissue scarring, kidney disease
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Our work is focused on examining the underlying mechanisms involved in rejection of kidney transplants which ultimately leads to failure of the transplant kidney. A better understanding of the immune, inflammatory, thrombosis and scarring processes will allow us to develop novel treatment strategies to improve the success of kidney transplantation.

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)?

Since the research programme is driven mainly by an integrated team of clinicians and scientist, all of our work is designed to be of benefit to our patients. It is realistic to propose that at least some of our work in experimental models may lead to novel treatment for patients. Furthermore, our results will be shared with scientific and medical communities through publication in scientific and medical journals and conferences. Progress in understanding of rejection of kidney transplants and development of new therapy will be shared with patient interest groups and the public. Potential clinical translation will be carried out through partnerships with the university, the funding bodies, research charities and the pharmaceutical industry.

What types and approximate numbers of animals do you expect to use and over what period of time?

Mice – 6600 /5 years (including 1600 for establishing genetically modified mice, by superovulation, generation of founder, embryo transfer and vasectomy, 4000 for breeding and maintenance of genetically modified mice, 1000 mice for induction and study of kidney ischaemia reperfusion injury). Rats – 9600 /5 years (including 1600 for establishing genetically modified rats, 5000 for breeding and maintenance of genetically modified rats , and 1000 for kidney ischaemia reperfusion injury, 1000 for preparation of donor rats for kidney transplantation, and 1000 as recipient rats for orthotopic kidney transplantation).

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Our protocols have been designed to reach a moderate level of severity at the maximum. We shall maintain the welfare of the animals by housing the animals in appropriately temperature regulated and hygienic areas, together with regular handling and inspection of the well being of the animals by investigators and experienced staff of the animal facilities, with regular advice from Named Veterinary Surgeons. Whole Body Irradiation: These animals will have a weakened immune defence system. Therefore, they will be protected from exposure to pathogenic agents by housing in barrier protection. Preventive antibiotics and specialised diet will be given at an appropriate stage after irradiation. Experimental models of ischaemia reperfusion of kidneys and rejection of kidney transplants: Pain will be prevented or minimised by the use general anaesthetics during the procedures and regular administration of analgesics after the operation. There is a minimal risk of induration, swelling and discomfort at sites of injection. Aseptic techniques will be used to minimise the risk of infection. Any location induration may be treated using topical treatment in consultation with NVS to alleviate symptoms. Wound dehiscence may be treated by re-suturing when required. The assessment of the experimental models is by measurement of physiological parameters (e.g. blood tests for chemistry and urine protein measurements) and assessment of kidney structure (histology) by microscopy collected at the end of the experiment. Therefore, the risk of complete kidney failure is minimised. In animals showing persistent weight loss up to 20%, they will be killed by terminal general anaesthesia or a Schedule 1 method to avoid suffering. Blood sampling: Blood sampling is needed to assess the biochemistry and immune status (e.g. antibody level) of the animals. The volume and frequency of blood sampling will be minimised following LASA guidelines. Any local bleeding after blood sampling will be controlled by local treatment (e.g silver nitrate or ferric chloride) or by applying digital pressure until bleeding stopped. Rarely, a haematoma (local collection of blood clot under the skin) may develop at the site of blood sampling. If this occurs, we shall seek the advice of the NVS and appropriate action will be taken. Further blood sampling will be delayed until the haematoma has resolved. General anaesthesia: The animals will be under constant monitoring during general anaesthesia to ensure a good level of anaesthesia and no adverse side-effects. If the animals developed an anaesthetic problem, they will be euthanised by

terminal anaesthesia. The animals may feel cold during the anaesthesia and also during the recovery. An external heat source will be provided during and after the surgery to maintain normal temperature. Saline will be administered under the skin to animals during and after surgery to compensate for insensible fluid loss. Any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health deteriorates significantly will be killed by a Schedule 1 method. End of the study: all the animals will be killed by terminal anaesthesia or a Schedule 1 method.

Application of the 3Rs

Replacement

The use of animals is essential for our work, since it is only by study of the intact animal that we will be able to work out how rejection of kidney transplant is caused and what therapeutic approaches might be of benefit. We also use cell culture experiments, where possible, to address specific questions about cellular mechanisms of disease, but this cannot reproduce the complexity of the whole animal.

Reduction

The models of ischaemia reperfusion of kidneys and rejection of kidney transplants which we have developed are generally extremely reliable and reproducible, so we can obtain scientifically significant results from small groups of animals. As stated above, numbers are also reduced by using in vitro methods where applicable.

The numbers of animal will be minimised by reducing variation of confounding factors (e.g. age, weight, sex, housing and husbandry), appropriate stratification for variables, and appropriate randomisation of animals between control and treatment groups, and blinding of researchers to control vs treatment arms to avoid any bias. Same sex of animals will be used in all the groups to avoid the confounding factor due to differences in response between male and female animals. We shall be able to design and analyse our experiments to be able to publish to the NC3Rs ARRIVE Guidelines.

Refinement

This application includes both rat and mouse models of kidney diseases, since each species has advantages for certain studies. Both mice and rats will be used in studying ischaemia reperfusion injury of kidneys. One advantage of mouse models has been the availability of a wide range of genetically modified mice which can be used to determine the effect of specific genes on the disease model being studied. However, there have been major advances in rat genetics over the last few years, and several genes involved in kidney disease in the rat have been shown to be relevant in the related human disease. Also, recent advances in techniques mean

that specific genes can be deleted in rats in order to study their relevance in particular disease models. We therefore wish to generate and breed genetically modified rats in order to investigate the more accurate models of human disease that can be produced in the rat as compared with the mouse.

Rejection of kidney transplants will be studied in rats only because of their larger size which is more appropriate for the surgical procedures of transplantation.

Because of the application of 3 Rs in all of our studies involving kidney disease in experimental animals, we assess the outcome of the experiments by using kidney histology or kidney function, at a relatively early stage of disease such that no animals suffer from the clinical effects of kidney failure. Any animal which becomes unwell during the course of the experiments, for any reason, will be humanely killed.

Project 18	Biochemical Pathways in Renal Fibrosis
Key Words	Renal, Kidney, Fibrosis, Therapeutic
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Chronic kidney disease generally proceeds to end stage renal failure, requiring replacement therapy in the form of dialysis or transplantation. The disease process is driven by fibrosis, and a key scientific goal is to understand the cellular mechanisms controlling fibrosis in order that new targets for therapeutic intervention can be identified and new drugs developed that can increase patient quality of life.

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 like benefits of this project will be a greater understanding of the pathways that drive scarring in chronic disease. We will be able to identify targets for therapeutic intervention. Retarding the scarring process in range of human diseases, including those affecting the kidney and lung, would represent a huge benefit to patient health and quality of life.

What types and approximate numbers of animals do you expect to use and over what period of time?

We would expect to use rats, mice and rabbits. Approximately 500 mice, 400 rats, and 600 rabbits 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 levels of severity? What will happen to the animals at the end?

The study will be employing models of renal scarring and the adverse events expected will be those associated with renal failure. These will include symptoms observed in man, uraemia, proteinuria, lethargy and mild symptoms of general poor health. We will be able to monitor renal function throughout the studies and ensure that no animals are exposed to more than moderate discomfort. Some models involve surgery and there may be mild discomfort immediately after surgery which will be managed with suitable analgesia. All animals will be terminated by schedule 1 procedure at the end of each study.

Application of the 3Rs

Replacement

The biochemical pathways involved in kidney disease are complex and, currently, there is no alternative *in vitro* technology that replaces the need to use animals since all components of the fibrotic pathway need to be present to accurately model the disease processes involved. Renal fibrosis is the result of a complex interaction between different cell types and signals, including inflammation, and the feedback and control systems of normal kidney function. At present these complex interactions cannot be recreated in either simple cell culture or by computer simulation.

Reduction

All of our studies will be preceded by small scale pilot studies to establish the magnitude of effect we will observe. This data will be used by us, together with our statistical advisor, to design studies that achieve significance with the most reasonable number of animals.

The effects of inherent variation will be minimised by randomising animal groups. In order to ensure statistical rigour at the analysis stage all samples will be blinded. All experiments will be carried out under conditions that will allow publication of results according to the ARRIVE guidelines.

The models used in this programme have been extensively characterised and based on these our previous earlier studies it can be estimated that , most experiments will involve group sizes of 4 to 6 animals, which will be large enough to provide statistical confirmation of meaningful treatment effects. A typical experiment will use approximately 30-40 animals. All animal numbers will be determined by power calculations based on analysis of small scale pilot experiments and we will seek to ensure that the least number of animals are used to obtain meaningful data and thus prevent unnecessary animal suffering.

Refinement

In the absence of useful cellular models we need to use animal models to accurately reproduce disease mechanisms observed in man. Mice, rats, and rabbits will be used in this project as these are the lowest animals on the evolutionary scale that offer models of renal scarring that reflect human disease. All animals will be subject to daily welfare oversight carried out by both experienced investigators and highly trained animal unit staff. We will have access to a named veterinarian to deal with queries regarding animal welfare, and providing additional oversight. We will be using predetermined objective scales of pain and discomfort (Appendix 1) and will ensure that severity limits are not exceeded.

Project 19	Regenerative medicine therapy for renal injury
Key Words	Regenerative medicine, cell therapy, kidney, injury
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Donated kidneys frequently have to be transported to the recipient. During this period of storage, the kidney will be damaged. Healthy kidneys can recover quite quickly, but kidneys from older donors often do not recover enough function to make them worth transplanting. Therefore potential donor organs are being discarded.

We aim to develop a new treatment which involves injecting cells isolated from the recipient's own body fat into the donor kidney to improve the kidney's rate of recovery. We have shown that these fat-derived cells can improve the function of an injured kidney in animals. We now need to establish the safety of this treatment: do the cells remain in the kidney or do they migrate elsewhere in the body? If they leave the kidney do they form tumours or damage other organs?

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

In the UK there are over 25,000 patients whose lives are being sustained by kidney dialysis. A kidney transplant would improve the quality of life and survival of these patients dramatically. However there is a shortage of donor organs which means that many patients will not receive a new kidney. One solution is to use 'extended criteria donor' (ECD) kidneys. These kidneys tend to come from donors >60 years in which there has been a delay between death and transplantation. Consequently, ECD kidneys are at greater risk of subsequently failing which makes them an unattractive option. If we can show that cells derived from body fat improve the recovery of injured kidneys without causing harm to the animal, then we can progress towards a

clinical trial in human volunteers. We are working with a transplant surgeon who will use an approved device to isolate cells and inject them into ECD kidneys in a ‘first in man’ clinical trial. Thus we anticipate that the proposed animal work will have direct clinical benefit in the next 5 years.

What types and approximate numbers of animals do you expect to use and over what period of time?

We have requested authority for 5 years. We will use rats for the planned experiments. The majority of the animals will be standard laboratory rats. We will also use rats that have been genetically modified to make the protein found in fireflies which makes them glow. If we inject cells from the ‘glowing’ rats into the kidneys of normal animals we can easily track where the cells go using an imaging device. In addition, we will use rats with a compromised immune system to test the safety of the cell treatment, as transplant patients will be taking immunosuppressant drugs which in turn could increase the risk of tumour formation. The total number of rats required = 1080

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The main cause of harm to a donor kidney in storage is a lack of oxygen. In order to recreate this condition in an animal model we will use an experimental approach called ischaemia reperfusion injury (IRI). This is performed under anaesthesia and involves temporarily clamping the blood vessels that supply one of the animal’s kidneys, so that blood flow is stopped. IRI causes damage to one of the kidneys; however because the second kidney remains intact the risk of death due to kidney failure is low. In order to introduce the therapeutic cells into the injured kidney, we need to inject the cells directly into the renal artery. This can be achieved in two ways; however both carry the risk of substantive blood loss as they involve creating a hole in a blood vessel under high pressure. We will perform a small pilot study to establish which method carries the least risk in our hands. Some risk remains that there will be blood loss which cannot be controlled during the operation. If that occurs, the animal will be killed without being allowed to recover from the anaesthetic. In some animals blood loss may be controlled, only for there to be subsequent internal bleeding upon recovery from the anaesthetic. Should this occur the animal will be killed. The cells that we will inject into the damaged kidney are expected to help repair the injuries, so their overall effect should be beneficial. If the cells leave the kidney we do not anticipate that they will cause any harm in normal rats. As transplant patients will be taking drugs to suppress their immune system, we will also establish what happens to fat-derived cells injected into rats with compromised immune systems. We know that transplant patients have a greater risk of developing cancer, so there is a risk that the injected cells may form a tumour in the immunocompromised rats. We will monitor the rats for up to 6 months to see if there is a greater risk of tumour formation; if this occurs the animal will be killed.

Application of the 3Rs

Replacement

Animals are necessary as we are trying to establish whether therapeutic cells injected into an injured kidney remain in the kidney or migrate elsewhere in the body where they could form tumours.

We will be conducting experiments on cells and isolated kidneys to answer some of our questions; however ultimately we have to test the safety of the cell treatment in a whole animal.

Reduction

The experiments are designed so that we are able to gain the most information from each animal. We have performed sample size calculations based on our experience with the model so that we know how many rats will be required to give statistically meaningful results.

Refinement

We will use rats in this study because they share similar physiology with humans and they are large enough to perform the surgery necessary to induce IRI. The IRI model allows one kidney to be damaged while leaving the other kidney intact, therefore the animal is unlikely to develop renal failure.

We will conduct a pilot study to identify the best method to inject therapeutic cells in the renal artery while minimizing blood loss, which is the biggest risk to the welfare of the animals.