Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 8

Projects with a primary purpose of: Basic Research – Urogenital and Reproductive System

Project Titles and keywords

1. **Cohesin and condensing function in mitotic & meiotic *cells***
    - Gene regulation, X chromosome, chromatin

2. **Amphibian embryos and ooctyes**
    - Axolotl, Xenopus, embryo, egg

3. **Genes and Hormones in Reproductive Health**
    - Androgen, testosterone, testis, Leydig, male

4. **Disorders of sex development**
    - Testis, Ovary, gonad, embryo, DSD

5. **Stability and dynamics of epigenetic information**
    - Epigenetics, chromatin, germ line, development, reprogramming

6. **Mechanisms of injury in polycystic kidney disease**
    - Kidney disease, polycystic kidneys, JAK-STAT

7. **Molecular basis of meiotic recombination**
    - Recombination, meiosis, PRDM9, fertility, speciation

8. **Microparticles and inflammation in chronic kidney disease**
    - Cardiovascular, kidney, endothelium, microparticles, phosphate

9. **Mechanisms behind early mouse embryo development**
    - Cell fate, mouse embryo, development, pluripotency, differentiation

Project 1	**Cohesin and condensing function in mitotic & meiotic cells.**	
Key Words (max. 5 words)	Gene regulation, X chromosome, chromatin	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	√	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The cohesin and condensin complexes are protein complexes that form rings around the DNA. The integrity of this ring structure is essential for their function in building chromosomes and controlling their proper segregation. The replacement of the genes coding for some of their subunits by a version coding for a modified protein that can be cleaved by the protease TEV, enable us to cut and abrogate the structure and function of these rings very quickly and efficiently. Using TEV-cleavable alleles of condensin or cohesin subunits we will address different objectives: 1/ Are these complexes required to ensure chromosome segregation during meiosis? 2/ The condensin ring is essential to build chromosomes but is it essential to maintain their structure? 3/ How do Condensin rings build chromosomes, when do they start to associate with DNA during the cell cycle in order to condense separately both copies of the genome? 4/ Using the TEV technology we also want to address the role of the regulatory subunits of the cohesin complex to understand how the ring is regulated, deposited and removed on the DNA both during	

	meiosis (in oocytes) and mitosis (in zygotes). 5/ Why do the germ cell version of the cohesin ring differ from the somatic ones? What are their specificities? By expressing germ cells version of the cohesin in somatic cells we will address the specificities of each subunit.
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)?	Errors in chromosome segregation result in aneuploid gametes containing too many or too few chromatids and this can have severe clinical consequences therefore we need a better understanding of the mechanisms of cohesin function in oocytes. Our work will provide fundamental insights into the mechanisms by which chromosome morphology is regulated in all eukaryotic organisms, which will in the long term prove of inestimable value in chromosome biology. Lastly, mutations in cohesin subunits have been implicated in a variety of tumours and greater understanding of cohesin function may therefore shed insight into aetiology of these tumours and provide new concepts for future therapies. Because condensin is related to cohesin, insight into the mechanism by which condensin holds DNA within chromatids together will help understand better how cohesin manages to hold sister DNAs together.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 50,000 mice over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	95% of animals are expected to be returned under a mild severity as these are to be used for breeding and schedule 1 with no adverse phenotype. The remaining 5% includes: a) mouse lines that have or may develop a harmful phenotype and these are recognisable and controllable. b) mice undergoing implantation surgical procedures. The majority of animals may be killed by a Schedule 1 method at the end of the protocol. For surgical protocols analgesic agents will be administered as required for these procedures.
Application of the 3Rs	
1. Replacement	There are no appropriate model systems for

State why you need to use animals and why you cannot use non-animal alternatives	mammalian meiosis and we are therefore continuing our studies using mouse oocytes. Moreover, mouse is the system of choice for mammalian genetics and engineering specified genetic modifications as it is the most well understood laboratory model for mammalian development. Yeast, insect cells and fly models are regularly used in my lab as an alternative to rodent use and my lab will continue to use these alongside the mammalian models. Where possible we use tissue culture models to address specific questions and we are continually trying to improve the potential of these strategies, We have replaced some mouse models entirely with tissue culture, but it is not possible to model all aspects of this process using a single cell type grown under artificial conditions in a tissue culture dish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible we obtain genetically modified/mutant lines from colleagues or internationally accredited repositories/suppliers, obviating the need to produce lines in house. We share vasectomised sterile male studs with other users, reducing the need to create and maintain duplicate animals. The animal numbers requested have been calculated based largely on experience and Mendelian genetics of multi-generation breeding programs as well as minimal maintenance of stock strains. Moderate strains are maintained wherever possible as heterozygote to wild-type crosses. For production of experimental homozygotes of moderate strains, heterozygote inter-crosses are used unless homozygotes are born at sub-Mendelian ratios, in which case heterozygotes are crossed to homozygotes. Mild strains are maintained wherever possible as homozygote inter-crosses except when homozygotes are infertile. Experiments are designed to use the minimum number of mice required for statistically significant results. Where appropriate we use cryopreservation of embryos and sperm to archive genetically modified strains that we have produced or imported, obviating the need to continually breed animals to preserve the lines.

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 most appropriate model for studying mammalian meiosis/mitosis because it is possible to introduce mutations into its germline and thereby analyse their effect on gamete cell cycle. We no longer require tail biopsy as the standard method to genotype our mice, there may be on occasion a necessity to take a tail biopsy, but these will be done on a case by case basis. All genetically complicated crosses are designed and maintained to produce the least amount of wastage as possible. We want to investigate the role of condensin during the chromosome pairing and homologous recombination processes in male spermatocytes using a strain in which the condensin can be cleaved by the TEV protease. This protocol will be carried out in 2 stages, 1. Pilot study to check effectiveness of TEV protease microinjection (< 30 mice to be used) 2. We will then continue to carry out the remaining necessary experiments if the pilot study works. This ensures procedure is effective before more animals are used. Mice known to develop tumours will have appropriate monitoring (for example weighing weekly) and any of the expected clinical signs presented will result in the mouse being humanely killed. Non-surgical embryo transfer is available and will be used where appropriate. Where we carry out ES cell/ CRISPR microinjection and culture until blastocyst stage we can transfer these via the non-surgical method. This method doesn't allow us to transfer anything pre-blastocyst and so stops us completely changing from surgical to non-surgical. For surgical procedures the appropriate analgesic agents and dose will be administered. For surgical protocols analgesic agents will be administered as required for the procedures.

Project 2	**Amphibian embryos and ooctyes**	
Key Words (max. 5 words)	Axolotl, Xenopus, embryo, egg	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Understanding how genes control the development of an embryo is a key objective of biomedical research because it can unlock the door to the development of therapies for an untold number of genetic diseases. However, the embryos of humans, and all other mammals, are difficult to obtain for both ethical and practical reasons, and because of this science relies on the embryos of related animals, such as amphibians. In contrast to the embryos of mammals, amphibian embryos are very large, available by the hundreds, and develop externally in water. Importantly, as well, recent research demonstrates that the development of embryos from axolotls, a specific species of salamander, is controlled by genetic mechanisms very similar to the mechanisms that control human development. This places axolotl embryos in a key position for unravelling many of the mysteries that underlie human development. We also have used comparisons between the development of embryos from axolotl and Xenopus as a tool to investigate how vertebrates evolved. Axolotls reflect the primitive state of amphibian evolution and their embryos can therefore be used as a start point for identifying the ancient mechanisms of vertebrate development. Xenopus, on the other hand, is a highly derived (unusual) species with traits that	

	are not found in any other lineage of vertebrates. Much is already known about how Xenopus embryos develop because they have been studied for decades in labs throughout the world. By investigating the response of axolotl and Xenopus to similar experimental treatments we have gained novel insights into the fundamental genetic mechanisms that govern vertebrate evolution, and this leads to an understanding of how humans evolved, and how the incredible diversity of in the animal kingdom emerged. In summary, the purpose of this license is to authorise the production of embryos for the License Holder's basic research programme. This is focused on understanding how the stem cells that form sperm and egg, known as primordial germ cells (PGC), or blood, known as haematopoietic stem cells (HSC) are produced during development. Also his group studies how the genetic mechanisms that control embryonic development evolved. In addition, the license will authorize the provision of a service to produce embryos for other groups, which will support research into a number of different questions about human development.
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 potential benefits of the research are the ability to identify the genetic pathways that cause defects in germ cell (sperm and egg) and blood development. The identification of these pathways can lead to targets for the development of therapeutic drugs. In addition, the comparison of axolotl and Xenopus embryos will greatly enhance our understanding of the genetic mechanism that control the evolution of vertebrates.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate the use of about 400 adult Ambystoma mexicanum (axolotl) and 50 Xenopus laevis adults over the lifetime of the project. We estimate the production of about 400 GAA axolotl embryos, from which no more than 50 GAA will adults will emerge over the five year licensing of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The procedures in this license are considered mild. They will involve the injection of syringe needle containing hormones (Human Chorionic Gonadotropin, HCG) into the dorsal lymph sac of Xenopus females, or into the dorsal musculature of axolotls, both males and females. After an individual procedure the animal will be returned to its tank and the next morning mild pressure may be applied to its

	abdomen to promote ovulation or the collection of semen. After these procedures the animal will be returned to the normal stock. It will be monitored for any adverse effects of hormone administration.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals develop in response to a highly ordered sequential activation of genes. While many aspects of genetics can be studied in artificial systems, like cultured cells, it is impossible to know, with certainty, the role of specific genes outside of the context of a naturally developing embryo. Since it is impossible to store the sperm and eggs necessary to produce embryos, the maintenance of adult animals in a breeding colony is inescapable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Adult animals will be maintained on a carefully monitored rotation that maximizes the efficiency of their probability for successful mating. From a single successful mating hundreds of embryos are produced, in amphibians. These embryos will be divided among as many different experiments as possible and statistical analysis will be employed to determine the minimum numbers of embryos required per experiment to produce scientifically relevant data. Together, these approaches will minimize the number of adult animals required to support the research project.
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 over a century scientists have used amphibian embryos as models to understand how vertebrates develop because the embryos are large and readily accessible, and so they are easy to manipulate for experimental purposes. Over the last several decades embryos from Xenopus laevis, a frog, have been used throughout the world to establish the basic parameters that control development of vertebrate embryos. However, we have spearheaded an effort to develop embryos from axolotls as an alternative to Xenopus, based on established evolutionary history of amphibians, which indicates that axolotls closely resemble the ancestors of mammals. We have demonstrated in many studies, in fact, that the same genetic mechanisms govern development of axolotl and human embryos, identifying axolotls as a uniquely useful amphibian model to understand what controls human development. No other animal model is suitable for these studies. Yet comparisons between axolotl and Xenopus embryos provide a

	unique insight into vertebrate evolution. Our research focuses on embryos, not adults, and so adult animals are not subject to experimental treatments. Further, in all instances embryos are collected before can eat food, so they have a minimal level of consciousness. Our breeding colony is maintained according the best practice authorized by the Home Office to ensure the highest quality of adult animal welfare.

Project 3	**Genes and Hormones in Reproductive Health**	
Key Words (max. 5 words)	Androgen, testosterone, testis, Leydig, male	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Androgens are steroid hormones (e.g. testosterone) that play a role in male traits and reproduction and have a life-long impact on the health of both men and women. Reduced androgen action in fetal life can have lifelong impacts on male health. Changes in androgen action in adulthood have also been linked to several diseases and conditions representing a significant health burden, including cardiovascular disease, diabetes and obesity, cancer, and polycystic ovarian syndrome (PCOS) in women. There is compelling evidence androgen concentrations decrease as men age, which has been linked to many chronic age-related conditions including cardiovascular disease, metabolic syndrome, poor healing, reduced muscle power and mobility, each of which represents a significant, and growing, health burden for the NHS. With an expanding ageing population in the UK, understanding how androgens promote health is essential component in developing future preventative measures and therapeutic options.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	We will develop a detailed picture of how androgen signalling controls development and function of the reproductive system.	

project)?	We will characterise the mechanism underlying control of androgen production. We will develop techniques to manipulate the system of androgen production for human and animal health benefit. Completion of these objectives will inform our understanding of how androgens control development and function, and reciprocally, how the body controls androgen production, and how these together impact health. It will also support the future development of refined therapeutics to support lifelong health and wellbeing.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years Mice: 20,500 Rats: 700
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?	For >80% of animals they will carry genetic modification and will be bred and maintained to specific ages before humane culling and post-mortem analysis. This will be mild severity. The remaining 10% of animals will undergo one of the following protocols: • Injection of hormones, cells, viruses, to inform our understanding of how the system works by modulating fertility. – moderate severity • Induction of cell death of specific populations of testicular cells. – moderate severity • Breeding of prematurely aged mice (due to mutations in key genes). – moderate severity • Breeding of genetically modified mice with impacted hormone production. – moderate severity. • Receive xenografts of gonadal tissue. - moderate Animals generated under a breeding protocol and which show no clinical signs may be transferred to one other protocol for analysis. All animals will be humanely culled 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	In the adult, development of the germ cells takes place within two highly specialised environments; the follicle of the ovary and the seminiferous epithelium of the testis. The morphology of the gonads found in mammals differs from that in insects and fish making

	these less suitable models. The development of the germ cells is dependent upon the support from somatic cells (granulosa, theca Sertoli, PTM Leydig cells). Although aspects of Sertoli and Leydig cell function can be studied using cell culture methods, full maturation of germ cells to functional sperm is not yet possible. The nature of the interactions between organs and cells needs analysis of intact tissues.. A computer model of the brain/reproductive system interaction will be used to replace animal use whenever possible. However, fertility is dependent upon not only the formation of mature eggs and sperm but the interaction between hormone control in the brain and reproductive system, some aspects of which can only be adequately studied in intact animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations will been used for all studies to ensure the least possible number of animals are used to be able to draw statistically valid conclusions from the research. General oversight of statistical analyses will be provided by our long-term collaborator who will also provide additional support with experimental design and statistical analysis. We have developed collaborations to exploit serendipitous models generated during our core research (providing additional scientific data output from each animal generated without extra breeding), and making use of the female animals generated as a matter of course by our studies. This also reduces further animal breeding by other researchers. The development and use of IV lentiviral vectors will reduce the need to breed and maintain transgenic mouse colonies. For ageing experiments, mouse models of premature ageing will reduce the need to breed and maintain additional animals as a back up for loss of experimental animals over the traditional 2 year waiting time.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Whilst the mouse and rat does not perfectly replicate every aspect of human testicular anatomy, the general anatomy, function and underlying mechanisms are largely the same, with loss-of-function mutations in androgen receptor for

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	example, producing similar phenotypes in both mice and humans. The combination of available technology and the similarity to the human makes mice the least sentient species we can use for our studies. We will use subcutaneous implants when relevant to reduce the need for repeated injection. Pilot studies of new techniques will be undertaken to establish the efficacy and impact upon animal welfare before larger studies are undertaken. We have chosen to use Lentivirus rather than adenovirus as we previously demonstrated adenovirus is immunogenic We submit experimental protocol forms for veterinary approval prior to the initiation of each experiment to ensure the most refined approach is always used. The use of well characterised ageing models will permit close observation of specific and well-described welfare issues these animals may face. Furthermore, compressing the time over which such symptoms are likely to appear means that close monitoring at defined periods will be certain to pick up any welfare issues that can be quickly addressed.

Project 4	**Disorders of sex development**	
Key Words (max. 5 words)	Testis, Ovary, gonad, embryo, DSD	
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)	Disorders of sex development (DSD) are a group of conditions affecting sexual development – the embryonic development of the gonads, internal and external genitalia - that vary in frequency and severity: mild cases such as failure of testis descent (cryptorchidism) can be as frequent as 1 in 250 births; severe cases, including ambiguous external genitalia (unclear whether the individual is male or female) and XY female development (complete gonadal dysgenesis), can be rare (less than 1 in 3000 births). At least some cases of DSD are caused by faulty genes (gene mutations). It may be that similar genes are involved in mild and severe cases of DSD, but impact in different ways depending on the genetic background of the individual and the nature of the gene disruption. But many cases of DSD remain undiagnosed: it is unclear why they occur but a genetic cause is likely. We are working with clinicians who are studying the genomes (DNA) of people with DSD in an attempt to find gene mutations that might explain the abnormality. Many mutations may be detected in an individual, but only one (or a small number) are likely to be responsible for the DSD in question. In order to help provide evidence that a particular gene is important in DSD, we are generating and studying mice in which we have	

	disrupted the same, or a closely related, gene. We are also using such genetically altered (GA) mice – also known as 'models' – to understand why the disruption to development of the reproductive organs occurs.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our main aims are twofold: i) by confirming that specific genes are required for sex development in the mouse, to contribute to the process by which clinicians are able to offer better genetic diagnoses of individuals with DSD – this is important for those individuals and their families; ii) to study the mechanisms by which genes control normal and abnormal sexual development in the mouse – the knowledge generated may be relevant to future aims of controlling the fate of stem cells for therapeutic intervention, of treating infertility and of regenerative medicine more broadly. It will not be possible to develop rational cell-based therapies of diseases without understanding in great detail how genes control the functions of the cells in which they are expressed and, when they go wrong, how they cause diseases. Such benefits accrue over different timeframes: we anticipate that generating and studying mouse models, which we have a track-record in doing successfully, will contribute to the identification of new human sex-determining genes. Alongside progress in human genetics and clinical research, there are tangible benefits to be had by studying sex development in the mouse. In the long run, we aim to contribute knowledge that will result in enhanced genetic diagnoses of DSD and future prospects for regenerative medicine.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use only mice – up to 50,000 in 5 years of research. The vast majority of our experimentation is performed directly on mouse embryos – the adult mice used are for breeding genetic alterations and for generating embryos. The numbers we need are mainly dictated by the very small size of the mouse embryonic reproductive organs – about the size of a human eyelash - which do not yield much tissue for study. We employ statistical tests in both the design and analysis stages of projects.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	The vast majority of adult mice used suffer no adverse consequences since they only carry genetic alterations – they are used in breeding schemes and for the generation of embryos. The embryos studied

level of severity? What will happen to the animals at the end?	have abnormalities, although these are often confined to the reproductive organs. Rarely, adult mice may have abnormalities and experience adverse effects – of moderate severity - for example, if a genetic alteration has an unpredicted consequence for another organ system apart from the reproductive tracts. Such mice would be very closely monitored and advice sought from the welfare officer (NACWO) regarding welfare to ensure the mice suffer only mild long-term or moderate short-term adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The reproductive organs – such as the testes and ovaries (gonads) – are highly complex, forming during embryonic development by the co-ordinated interaction of different types of cells over several days. This requires closely regulated cell behaviour – and it is genes that are responsible for this. Sometimes genes fail to perform their task – most often when they contain errors (mutations) that prevent them from working properly. In order to understand how genes control such complex cellular tasks, we need to study the consequences of gene failure in the whole organ. It is not yet possible to generate the cells we need to examine just by culturing them in a dish. Moreover, studying the consequences of gene failure in individual cells, or even types of cells, in a laboratory test-tube or dish does not give us the information required in order to understand the multi-cellular disease process that can occur in the growing embryo, which can result in birth defects. Mice are closely related to humans – in terms of genetics, anatomy and physiology – and provide the best way of modelling disease processes that occur in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have monthly planning meetings to ensure that breeding strategies generate only the genetically altered (GA) animals required. Numbers are calculated based on statistical tests that we use – with advice from a professional statistician. We cryopreserve any lines that are no longer required. We only rarely generate our own GA lines – and the use of new genome editing techniques (CRISPR/Cas9) should permit the use of fewer animals when compared to traditional stem cell-based methodologies, and the generation of mice that truly model the genetic alterations found in patients.

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.	Non-mammalian vertebrates are not suitable for our studies given the differences that exist between their reproductive biology and that of humans – mice are the simplest mammal that can be used. Our interest is primarily in the genetic causes of birth defects that arise initially during the embryonic period. The vast majority of our experimentation is directly on embryonic tissue, which is generated by breeding adults that only carry genetic alterations and therefore do not suffer adverse effects. If we need to examine adult mice we will try to restrict the genetic alteration to the reproductive organs, if possible, by using tissue-specific conditional gene ablation.

Project 5	**Stability and dynamics of epigenetic information**	
Key Words (max. 5 words)	Epigenetics, chromatin, germ line, development, reprogramming	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	At the beginning of the development the cells of early embryos are uncommitted and able to differentiate to give rise to any cell type. This capacity is gradually lost as the cells undertake cell fate decision. The memory of all these decisions is written in the form of chemical modifications of DNA and proteins associated with DNA (such as histones) as well as in the structural configuration/folding of the genetic material. Our research aims to understand how this additional (epigenetic) information can be removed in order to change the cell fate.	
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)?	Detailed understanding of how cells can erase epigenetic information globally will allow us to reprogramme somatic cells to pfuripotent stem cells with high efficiency. This knowledge will also open a possibility of a directed efficient transdifferentiation (changing of a cell of one type into a cell of another type). These approaches provide future avenues for the field of regenerative medicine. Additionally, our work will contribute to better understanding of the molecular processes responsible for appearance of germ cell tumours. Last, but not least, our work on early preimplantation embryos is contributing invaluable knowledge towards improvement of handling and culture of human gametes and embryos	

	during IVF and other Assisted Reproductive Technologies (ART).
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 32000 over period of 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In most cases we sacrifice adult mice (mostly pregnant females) to isolate early preimplantation embryos or foetal germ cells at various developmental stages. We also generate genetic loss of function mouse mutants, whereby genes of interest are deleted specifically in the germ line. Such genetic manipulation is expected to cause sterility (germ cell loss) or, alternatively, early embryonic lethality due to altered function of gametes. Such phenotypes are not expected to cause any significant suffering to animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Up to date there is no in vitro system that would recapitulate global epigenetic reprogramming to the same extent as observed in vivo during embryonic development. Wherever possible, we will use in vitro systems — such as mouse pluripotent embryonic stem cells (mESCs) or mouse pluripotent embryonic germ cells (mEGCs) to replace the animals. In addition, we have recently established an in vitro differentiation protocol that allows us to generate mouse PGC (primordial germ cell) like cells (PGCIC5, Hayashi et al, Cell 2011). Although none of these in vitro systems can fully recapitulate the reprogramming process that occurs in vivo, they can be used to study specific biochemical aspects.
2. Reduction Explain how you will assure the use of minimum numbers of animals	1) Wherever possible we will use the above mentioned in vitro systems. 2) We have optimised our methods in order to be able to carry our analyses on the minimal size samples (we have developed an ultra-sensitive LC/MS which requires only hundreds of cells for analysis instead of tens of millions of cells typically used for these analyses; we are using single cell RNA sequencing etc...). 3) We are working in collaboration with our experienced transgenic team. Their expertise limits the

	numbers of animals required to generate new transgenic strains.
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 timing and occurrence of epigenetic. reprogramming during mouse embryonic development has been well characterised .It has been recently shown that mechanistically many aspects of this process are conserved between mouse and human (Smith et al, Nature 2014, Tang et al, Cell 2015; Guo et al, Cell 2015; Gkountela et al, Cell 2015); it is thus conceivable that our findings regarding mechanisms of cellular reprogramming will be directly applicable to human cells for future use in regenerative medicine. All animals will be housed in groups where possible with appropriate environmental enrichment and fed according to current institutional ‘best practice’.

Project 6	**Mechanisms of injury in polycystic kidney disease**	
Key Words (max. 5 words)	Kidney disease, polycystic kidneys, JAK-STAT	
Expected duration of the project (yrs)	3 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)	Polycystic kidney disease is a very common condition but currently remains without a cure. Our purpose highlighted by our objectives are to better understand polycystic kidney disease and to develop novel therapies to cure it. We aim to do this by inhibiting the JAK-STAT pathway.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our research and aims has two main purposes firstly to control disease and find new therapeutic targets and secondly to better understand the pathophysiology of polycystic kidney disease. Both of these aims will result in better quality of life for patient suffering with PKD in the future. Patients with PKD currently account for 1:800 therefore a significant number of people are likely to benefit from our work. Some of our results may then extend to other renal proliferative disease such as renal cancer.	
What species and approximate numbers of animals do you expect to use over what period of time?	We are planning to only use mice and various genetics backgrounds will be used. We estimate to use a total of 1600 animals over the 5 years of this licence. We will breed animals on protocol 1 and experimental animals will transfer to protocols 2 and 3.	
In the context of what you	We study polycystic kidney disease which is a	

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?	genetic disorder affecting mainly the kidneys. Late stages of PKD lead to renal failure however we will closely monitor our mice and not allow them to progress to failure. Mice bred on protocol 1 will be used in protocols 2 and 3 and all animals will be sacrificed by the end of protocols 2 and 3.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have previously carried out extensive experimentation using cultured human cell lines, however, experiments in cells cannot wholly mimic whole body or organ systems and the disease that affects them. Thus ultimately it is only possible to test our hypothesis about what affects polycystic kidney disease progression using mouse models of human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before performing any experiment we read all relevant published papers to avoid unnecessary repetition. We then design our experiment and talk to our statistician colleagues to get an statistical estimation of minimum numbers of mice required to achieve a meaningful result (statistical significance). We are also in constant contact with animal technicians and have found ways to optimise breeding to avoid unnecessary breeding of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We study a renal disease with a known genetic cause; therefore we only use mice relevant to this genetic disease. These mice develop polycystic kidney disease with accompanied decline in renal function. Disease develops rapidly in these genetic models of PKD, and therefore most protocols will be completed before the age of 8-10 months. Mice will not be kept beyond the age of 12 months. In addition we have endpoints based on biochemical parameters that where possible will end a procedure before the onset of clinical evident disease.

Project 7	**Molecular basis of meiotic recombination**	
Key Words (max. 5 words)	Recombination, meiosis, PRDM9, fertility, speciation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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)	Recombination is a biological process that creates genetic diversity in all living organisms by forcing the exchange of DNA between chromosomes. Important questions such as how and why recombination vary between individuals in the human population are still unanswered. PRDM9 is the molecule that initiates this process by positioning the recombination machinery at the correct sites along the chromosomes. Interestingly, in the mouse, variation in PRDM9 correlates with variation in recombination and levels of fertility between certain strains. The main objective of this project is to study recombination and its links to fertility, using the mouse as a model system. Specifically, we aim to determine how PRDM9 controls the recombination machinery to ensure recombination occurs correctly.	
What are the potential benefits likely to derive from this project (how science could be	This project will greatly advance our knowledge and understanding of the recombination process and the key molecules involved. Importantly, recombination	

advanced or humans or animals could benefit from the project)?	errors can result in chromosome abnormalities in reproductive cells, which are the major cause of pregnancy loss in humans. They are also responsible for a number of disorders with body malformations at birth and mental impairment. 15% of the world population is affected by fertility problems; however, the molecular and genetic defects involved are mostly unknown. By characterising the molecular determinants of recombination, this project may help diagnosis and open new treatment options for infertility.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5000 mice will be used in this project over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals used in this project are only expected to experience mild adverse effects, with a small proportion experiencing moderate effects. The genetic modifications chosen are not harmful and will only reduce the fertility of the animals. In rare occasions, mild pain and discomfort could be experienced by the very small number of animals (5%) who will receive drugs to control the expression of some of these genetic modifications. All animals will be carefully monitored for additional unpredicted effects. Genetically modified mice will be killed at the end of the studies for tissue analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We intend to continue to use non-animal *in vitro* mouse and human cell systems where important factors of recombination can be artificially expressed and some aspects of their function studied. However, these artificial systems lack the specialized factors only expressed in the male and female reproductive tissues. Until appropriate cell systems are developed, recombination can only be studied in the whole body where the correct molecular, cellular, and physiological environment is present.
2. Reduction	We will reduce the number of animals by testing all reagents and methodologies first in non-animal *in*

Explain how you will assure the use of minimum numbers of animals	*vitro* cell systems if possible. They will then be validated in a small number of animals to estimate the appropriate number to be used to achieve the results needed. Once determined, we will carefully control the breeding to avoid excessive and unnecessary production of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the most widely used animal model in research to study important biological processes in human health and disease. Due to similar genetics, developmental biology and physiology between mice and humans, genetic manipulation in the mouse can be used to identify disease mechanisms and develop treatments for humans. Based on these principles, we have chosen to use the mouse as our experimental animal model to study recombination and its role in fertility in mammals. Importantly, infertility naturally occurs in males of certain mouse species, recombination events are easy to detect between very distinct mouse species, and a number of mouse mutants already exist for the recombination machinery. For all these reasons combined, the mouse represents the most appropriate model for this research and its relevance to infertility in humans. No harmful consequence is expected from the genetic modifications studied in these animals besides a reduction in fertility, and possibly a mild discomfort during transgene induction. However, the development of additional unexpected adverse effects will be continually monitored. Pain and discomfort to the animals will be kept to a minimum throughout the course of the project by regularly reviewing and improving the care and protective measures with the animal welfare staff. For instance, alternative methods of drug administration to injection will be used in pregnant mice.

Project 8	Microparticles and inflammation in chronic kidney disease
Key Words	Cardiovascular, kidney, endothelium, microparticles, phosphate
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):

In patients or animals with kidney failure, disease of the heart and blood vessels (cardiovascular disease) is the commonest cause of death, partly because of increased blood clotting. In kidney disease, small particles called microparticles break off the surface of the endothelial cells which line the inside of the blood vessels. These microparticles then circulate in the blood. This is potentially dangerous because some of these particles can increase blood clotting. Nearly all patients with advanced kidney disease have an abnormally high concentration of phosphate in their blood, and this may be one of the important factors which increases microparticle formation.

We aim to study how phosphate exerts this effect, and use this information to design new ways of preventing this problem. We aim to do this in 2 ways:

1) Firstly (without the use of animals) by adding phosphate to human endothelial cells which have been cultured in the laboratory, to see how this affects signals within the cells which trigger microparticle formation

2) Secondly by changing blood phosphate levels in rats with kidney disease (by varying the amount of phosphorus in their diet) to see whether microparticle numbers change, and whether signals in their endothelial cells are the same as those in cells cultured in the laboratory.

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)?

Chronic kidney disease is a major world health problem, currently estimated to affect ~5-7% of the world's population, and carries with it an 8 - 10 fold higher risk of death from cardiovascular disease than in the general population. Therefore even marginal improvements in therapy may result in considerable human benefit in this large patient population. It is hoped that this project will help to identify a key step in the development of cardiovascular disease in kidney patients at which therapy aimed at reducing the concentration of phosphate in the patients' blood may be particularly effective.

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

Rats Approximately 135 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?

An important feature of the early stages of kidney failure in humans and animals is that considerable damage to these organs can occur without significant symptoms being felt. This means that, with care, a limited degree of kidney failure can be induced in rats for medical research purposes without inflicting major suffering. To achieve this in the rats, under general anaesthetic the minimum amount of kidney tissue necessary to induce the required degree of kidney failure is removed surgically under aseptic conditions. For comparison a similar group of rats are anaesthetised and receive a surgical incision and the outer capsule of the kidney is separated from the rest of the kidney, but they are then sewn up again without removal of any kidney tissue. This is referred to as a "sham operation" and is important to eliminate any effects in the experiment that arise from surgery itself rather than from kidney failure. The animals are then allowed two weeks to recover from the surgery. During this time any discomfort arising from the surgery is treated by administration of analgesic. After two weeks the animals recover from the surgery and achieve a stable state (with some weight loss but otherwise without any detectable sign of discomfort) resembling human chronic kidney disease. They are then given several weeks of a modified diet containing high or low phosphorus content until the expected change in the concentration of microparticles in their blood is observed in small blood samples drawn from their tail vein. At that stage, to minimise the duration of the period of chronic kidney disease, the animals are then killed. Blood and tissue (including major blood vessels) are removed to measure the

effect of the different dietary phosphorus loads on the microparticles and blood vessels. If during this project we identify compounds or changes in diet which reduce the cardiovascular effects of chronic kidney disease, we will test their effectiveness in this rat model of kidney disease.

Application of the 3Rs

Replacement

Why animals have to be used

For several years, work on this problem in this laboratory has been carried out (without directly exploiting animals) by studying endothelial cells grown in culture dishes. While valuable information has been obtained in this way, this may be an imperfect model for the behaviour of human endothelium in patients with chronic kidney disease (CKD) for at least 2 reasons:

1) Interactions between endothelium and other cell types may be important during CKD, especially the production of pro-inflammatory molecules by a number of different cell types which may then radically alter the behaviour of endothelial cells.

2) The environment of endothelial cells in a CKD patient depends on a complex series of chemical changes brought about by kidney failure which can only be partly re-created with cells in culture dishes.

Therefore, to prove conclusively that high blood phosphate is causing damage to endothelium in blood vessels during kidney disease requires direct measurements on blood vessels and blood samples obtained under rigorously controlled conditions of high and low phosphorus diet in a living animal. This cannot be done safely in humans with kidney disease.

Reduction

The number of rats used in this study is the minimum number calculated to be necessary to allow clear detection of the expected effects that are to be measured in the blood and tissue.

The number of rats required is further minimised by running pilot studies using small numbers of rats (to detect and eliminate technical and animal welfare problems) before the main study is performed with a larger number of rats.

Refinement

Rats are used in this study, because experience over a number of years has demonstrated that this surgical method for inducing a slowly progressing form of chronic kidney disease yields a model of the disease which leads to changes in the heart and blood vessels which mimic closely the changes observed during human kidney disease. By careful monitoring of the body weight, food intake and behaviour

of the animals after the surgery, and by running the study for the minimum period of time, this can be achieved with as little suffering as possible to the animals.

Project 9	**Mechanisms behind early mouse embryo development**	
Key Words (max. 5 words)	Cell fate, mouse embryo, development, pluripotency, differentiation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this work is to provide an understanding of early mammalian embryo growth and development. We will study embryo development during two main phases. The first phase, pre-implantation, begins with fertilization of the egg by the sperm, The fertilized egg then divides sequentially over the next few days as the embryo travels along the oviduct towards the uterus. After 4.5 days, the embryo is a free-floating, organized ball of cells called a blastocyst. In the second phase, pen- and post-implantation, the blastocyst embeds itself into the mother's uterus and initiates growth and major re-organisation to establish future body. We will use mouse embryos as well as embryonic stem cells, cells isolated from the early embryo and grown in culture, to study cell dynamics and mechanisms that control these stages of	

	development. In particular the following questions will be addressed: 1. How do cells decide their fate to establish the blastocyst structure? 2. What genetic changes occur within specific embryonic cell types as the embryo develops and cells differentiate? How do these changes influence the ability of cells to differentiate into specific cell types? 3. What are the cellular and molecular reorganizations that occur in each tissue at the implantation stages? 4. What happens when embryo repair after perturbations? 5. How does the embryo become reshaped at the time of implantation to establish the fetus?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding early embryo growth and development at implantation stages may help improve the success of IVF techniques. Determining the mechanisms underlying the first cell differentiation events in pre-implantation development is fundamental for defining the optimal conditions for IVF and selecting viable embryos for further development. Blastocyst stage embryos are unique in that their cells can give rise to all adult cell types. These 'stem cells' can also be isolated and grown in culture, retaining the ability to differentiate to any cell type. Extending our knowledge of stem cells might also advance their use in regenerative medicine. This is the process of replacing or regenerating cells or in injured or diseased tissues! organs to restore normal function. Given their regenerative abilities, embryonic stem cells are potentially very important in such therapies. Studying stem cells enables us to determine the unique properties of these cells, and how they differ from differentiated cell types.
What species and approximate numbers of animals do you expect to use over what period of time?	All our research is carried out on very early mouse embryos. We expect to use an average of 114 female mice/week as a source for embryos during the 5 Years of the project. We will also estimate to have an average of 150 cages/week of transgenic mice during the 5 years of the project. Therefore, we will need 29 595 females for super ovulations. We also predict

	that we will use 23 000 mice for generation and maintenance of the transgenic and knockout lines required for this Study.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the work we propose will be carried out on embryos and so suffering to adult animals is minimal. We will study development of embryos in culture ex vivo, for example, by imaging which does not cause any adverse effects to mice. Embryos will be recovered from mice that have been either ovulating naturally, or that have been induced to super ovulate by provision of hormones, and mated with males and finally humanely killed. In experiments in which a specific genetic alternation has to be made provision of agent such as doxycycline or Tamoxifen will be given in drinking water. Moderate adverse effects may occur such as imbalance of the intestinal flora, resulting in diarrhoea and weight loss. If these cannot be ameliorated then the animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The intrinsic purpose of this study is to understand what happens with the embryo before and after implantation into the uterus. During that time the embryo undergoes huge changes in a short space of time. The mouse is a particularly powerful model to study this stage of development due to the ability to exploit the vast knowledge of mouse genetics and by genetically modulating gene function. This is a critical molecular tool in determining gene function. We will also explore the extent to which specific developmental processes we study can be substituted using Embryonic Stem (ES) or induced Iuripotent item (iPS) cells differentiating in culture. This replacement has been also used in the lab in the past and we will continue to do so whenever possible. Cultured cells cannot however give any information about spatiotemporal regulation of gene expression and the spatial interaction of tissues at specific developmental stages to mediate fate decisions in the developing embryo. Thus mice are an absolute

	requirement as experimental material.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all experiments we will first perform pilot experiments using a small number of animals and embryos to refine the experimental procedure and design. We will then expand the experiment to use the minimum number of animals required for statistically significant information. The utilization of genetically modified mice that express a fluorescent signal in specific cells of the embryo greatly facilitates our observations of spatial events in the embryo and means that we can use fewer embryos to obtain significant results. We also routinely use super ovulation, a method of ovarian hyper-stimulation, which increases the number of eggs available for fertilization, to increase the number of embryos that can be recovered from one mouse. All these procedures allow us to reduce the number of animals required for experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse provides a best model to understand development of the human embryo. The advantage of the mouse model for such studies is that mice are also the only species on which, for technical reasons, the genetic manipulations that are essential for this work are capable of being carried out. For technical reasons, mice are the only species where such manipulations are possible. Importantly, during the course of current project licence we have established a new system that enables for the first time filming and experimentation on mouse embryos developing from the pre-implantation to the post- implantation stage in vitro. With this new method in hand we can now significantly reduce the need to transfer embryos to the mice to study their subsequent development.