Private & confidential: *Please be aware that the contents of this form may be made public resulting from the "Freedom of information Act". Personal details will not be released.*

G. Non-Technical Summary (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Evaluation of potential for human embryos and embryonic stem cells
Key Words	Pluripotent stem cells, preimplantation-embryo, kidney cartilage, implantation,
Expected duration of the project	year(s) 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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(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);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Aim: understanding of how early embryos develop and implant in the uterus. We use the mouse as a model as it is a mammal like humans with a similar reproductive process and its genetics are well established. We apply our findings to human reproduction and development e.g. to determine if critical components are present in human embryos (obtained after informed consent from the IVF lab) or whether experimental interventions alter key implantation stages.

Aim to understand normal and abnormal human tissue development

We use human stem cells to model cell specialisation and test if findings from mouse are relevant to human development. We make tissues form human stem cells made from patients with genetic disease. Because we can make specialised tissues from these stem cells we can test what goes wrong in the development of these diseased human tissues. We aim to test these in animals since the tissues do not develop fully in the dish, they need the body environment. We make human stem cells become a variety of tissues of the body in order to compare regular development with that from cells with genetic mutations. We are studying diseases of cartilage and bone as well as of the kidney, nervous system and vasculature.

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)?

By understanding the process of early development and implantation we hope to develop therapies to overcome some rare incurable human diseases. We do this by developing mini tissues in a dish from stem cells generated from patients who are healthy or have mutations found in patients with such rare diseases. We have developed such tissues for kidney the blood vessels, cartilage and bone. By studying what goes wrong in the diseased tissue we identify targets for drugs and can test these. However the tissues only fully develop in the body so we need to test them by implanting in an animal. We can generate cartilage cells from human stem cells and implant them into small defects in the rat knee joint to show they can repair these defects. Thus we produce preclinical data to help us to develop a new safe and efficacious cell therapy for use in humans who have e.g. Osteoarthritis or sports injury.

We aim to improve ongoing pregnancy rates in IVF/ART and determine the long term effects of these procedures by optimising embryo development and embryo- maternal communication. This includes identifying druggable pathways that enhance or accelerate embryo implantation, or stressors that should be avoided in the IVF laboratory.

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

780 mice over 5 years 200 rats 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?

We will give natural reproductive hormones by injection with little adverse effect except soreness at injection site. Embryos will be collected from animals after humane killing. To assess embryo implantation, embryos will be transferred to non-pregnant recipient animals by injection into the uterus during anaesthetised surgery. Some discomfort will follow surgery which will be managed with analgesics, and

infection is very rare. Implantation of cells to test if they can form a benign tumour containing a variety of tissues tells us if these cells are able to make specialised cells. This will give some discomfort following surgery (minimised by use of a syringe for application where possible) which will be mitigated by analgesics, but infection is very rare. Animals will develop the benign tumours which do not invade and spread and are small, but if cysts develop and cause discomfort then animals will be humanely killed. Similar surgery will test if stem cell derived tissues can form mature cells of different tissues when implanted under the skin. There is little discomfort but animals will be monitored as for benign tumours. Cartilage progenitors will be put in a small abrasion in a hind limb joint of a rat to test potential for joint repair. This will give some discomfort following surgery mitigated by analgesics, but infection is very rare. The rats may have a stiff joint the next day following surgery. All animal will be killed humanely at the end of the experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We cannot study the process of embryo-implantation into the uterus in the human for ethical reasons. We use the mouse because a mammal is essential for investigation of embryo implantation into the uterus; it provides a model similar to human. The mouse is considered to be the least pain-sensitive mammal. There are no entirely satisfactory models in a dish for embryo implantation, so a combined approach using research outside the body (in vitro) and with animals is essential. Data on implantation obtained from mouse embryos in a dish (our main experimental model) must be checked in animals. We use embryonic stem cells (hESCs) to study how embryonic cells become specialised, avoiding animal use and comparing to human and murine preimplantation embryos in vitro. In vivo protocols are only used when there is no alternative, such as to validate data obtained in vitro. This is essential because we can only examine the earliest stages of tissue development/implantation in the dish.

We use the gold standard method for testing human embryonic or similar stem cells for potential to form specialised cells and tissue formation. This is the benign tumour assay in mice, which requires implantation into a mouse because the cells cannot reproducibly produce tissues in a dish, or in alternative models (e.g. zebra fish as an alternative is unsuccessful).

For preclinical research on hESC-derived chondrocytes it is essential to test the cells for ability to make true knee-type cartilage in the joint. There is no reproducible model in a dish to test this. Thus we need to use the rat cartilage repair model, the rat being a mammal with sufficient body weight and depth of cartilage to provide a valid initial test system (not the case for mice). We cannot test human cartilage cells in non-mammals to give meaningful data on joint repair.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Where possible experiments are undertaken with stem cells thus avoiding mouse embryos. This is not possible for embryo implantation. Here we carry out most studies with very early embryos: a ball of < 100 cells together with cell lines or cells from the lining of the human uterus, in a dish. Only validation experiments will be done using mice.

Embryos: We will use the minimum number of female mice to generate the minimum number of embryos needed for statistical significance in implantation studies in culture.

Benign tumours: are produced from stem cells by 4/ 5 mice injected. Therefore, a minimum to assure tumour formation of 4-6 animals are used for each human stem cell lines tested. Whole animal non-invasive imaging for teratomas will increase the information/animal and reduce animals used in future.

Cartilage repair; Cartilage repair from our cells is seen in around 75% of joints. Therefore, we need a minimum of 11 animals at each time point to monitor the process of repair. There is no reliable in vitro assay to assess cartilage repair. We have also introduced various whole animal imaging steps to increase the information obtained from each animal and reduce animals further.

We do extensive monitoring of all cells and embryos in a dish before use in animal models to ensure reproducibility and avoid animal wastage. We use our extensive experience to determine the number of animals we use is kept to a minimum. We will evaluate all experiments immediately on completion so that we do not use more animals then necessary.

Refinement

Explain the choice of animals 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.

Refinement

We use the mouse for studies on reproduction because a mammal is essential for investigation of embryo implantation and this provides a model similar to human. The mouse is considered to be the most tolerant, least pain-sensitive mammal, with the best understood genetics.

For surgical procedures, we use general anaesthesia in purpose built operating theatres with best-practice operating techniques to avoid infection and surgical complications. We apply pain killers to minimize postoperative discomfort.

The cartilage repair experiments are done in rats since the rat is considerably more weight- bearing than mice and the joint in the mouse is too small to reliably confirm joint repair especially as this needs to scale-up for human joint defects which can be up to 8mm across. The rat work is designed to be a necessary step before larger animal models (e.g.minipigs) and phase 1 human clinical trials in future work. We are developing imaging techniques to monitor cartilage repair in situ which will allow us to i) visualize repair in animals harmlessly; ii) monitor the time course of repair; iii) use fewer animals since animals can be viewed at different times and killed humanely after a final scan.