<table>
<tr><td>Project Title (max. 50 characters)</td><td>Designing a localised drug delivery system to treat endometriosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Endometriosis, gynaecological, infertility</td></tr>
<tr><td>Expected duration of the project (yrs.)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Endometriosis is a gynaecological disorder which affects 10% of women of reproductive age and causes symptoms of chronic pelvic pain and infertility. Current treatments are associated with an unacceptable level of side-effects or impairment to fertility. New management approaches arise from understanding the mechanisms that enable endometrial cells to survive in unwanted locations. We aim to gain improved understanding of the mechanisms involved in endometrial cell implantation by different stages of disease progression. We also aim to investigate novel treatment strategies by using an animal model which is well validated against the human disease.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>It is evident that there is a need for further refinement of preclinical animal models to ensure maximum resemblance to human disease. We have to design our preclinical studies in such a way that enables the most rigorous drug efficacy testing to enable clinicians to make informed decisions regarding the reliability of transferring the therapeutic to humans.

Benefits of this work are:

1) advancing our scientific understanding of mechanisms which aid survival of endometrial cells at unwanted locations
2) refinement of animal models enabling more robust translation to clinical research
3) validation of the animal model to human disease
4) potential validation of imaging techniques</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

	applicable to endometriosis models 5) proof-of-concept studies that drive new targets towards clinical development
What species and approximate numbers of animals do you expect to use over what period of time?	Approx. 1500 female 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?	In a typical experiment we will surgically induce endometriosis in mice then treat them with various therapeutic approaches and monitor endometrial lesion response by direct calliper measurements or imaging techniques. Developing these models and using them for drug testing is labelled moderate severity. Endometriosis is induced by transferring non-fluorescent/ fluorescent endometrial tissue from donor animals to recipient mice via surgical procedure. Adverse effects due to endometriosis initiation are rare, but could include infection, pain or introduction of mouse pathogens. These effects are all countered by good aseptic technique, use of analgesics and screening of murine cell lines to ensure no pathogens are present. Surgically introducing endometrial tissue will control where lesions will develop to minimise the probability of them disrupting other biological systems and affecting animal behaviour. Imaging is incorporated to monitor endometrial lesions in a non-invasive manner and to provide a reliable tool for assessing treatment efficacy. Pilot studies will be used to determine model progression and identify early indicators of decline in well-being that can be used as end- points for subsequent studies (e.g. general loss of condition or weight). Overall, wellbeing is monitored using a Health Score Sheet recording system that uses measures such as weight, appearance and behaviour in addition to evaluation of lesion burden/appearance to give an assessment of mouse welfare. This enables early interventions before substantial deterioration is observed and allows the accrual of information used to improve the overall process in subsequent studies (e.g. recognising the timeframe over which endometrial lesions develop and increasing frequency of monitoring accordingly,

	identification of clear end-points). Through recording and monitoring we endeavour to ensure adverse events are minimised and moderate. In all cases the animals are culled at experimental endpoint.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We cannot yet fully model the complex peritoneal environment, signalling processes or cellular interactions which are crucial for endometriosis development in humans. Practically and ethically we cannot test in the clinical setting and therefore must use species with physiology that best represents what we would expect to find in the human diseased state. Before initiating *in vivo* studies we will undertake comprehensive studies *in vitro* using endometrial cell cultures derived from mice and humans to ensure the clinical validity of the model. Drug efficacy studies will be completed in these cell systems coupled with *in silico* evaluations of drug/target interactions and pharmacokinetics to enable informed judgements regarding which therapeutics should be tested.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce inherent variability by using adult, same sex, age matched animals. We treat animals bearing endometrial lesions of equivalent size, which markedly improves the uniformity of response, thereby requiring fewer mice. We will use a randomised design with group sizes determined by power analysis using freely available software (SISA, http://www.quantitativeskills.com/sisa/). Correspondence with expert biostatisticians and researchers who have developed an animal model of endometriosis at the University also contributes to fewer animals. Where appropriate imaging techniques will be employed to enable multiple assessments to be made in the same mouse, pre and post therapy. Often applying a sequential study design in this case as it may not be possible to image all mice required in a single imaging session. The initiation of endometriosis is staggered over time, with generally 4 mice imaged per day. This is repeated until the experimental group sizes are achieved. Archived imaging data is used to refine sample size calculations enabling a reduction in group sizes.
3. Refinement Explain the choice of species and why the animal model(s)	Although primates spontaneously develop endometriosis identically to humans the least sentient animal sufficient for this research will be

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.	used. Mice will be artificially induced with endometriosis because they develop lesions which most closely resemble human disease compared to other rodent species. They have the lowest neurophysiologic sensitivity in which well-characterised models of endometriosis already exist. Correlative studies reveal mice and women share similar genetic alterations and sensitivity to hormones involved in endometriosis, indicating the potential for inter-species translation of results. To minimise welfare costs we closely monitor mouse wellbeing and identify appropriate intervention points from pilot studies. Following surgery animals will be closely monitored and individually housed in temperature controlled incubators with more accessible food until fully recovered. Imaging techniques will be employed to visualise changes over time and determine when best to analyse by histology.