Home Office

NON-TECHNICAL SUMMARY

# Assessing Novel Treatments for Endometriosis

**Project duration**

5 years 0 months

**Project purpose**

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

**Key words**

Endometriosis, Preclinical model, Fertility, Drug therapy, Inflammation

| Animal types | Life stages |
|---|---|
| Mice | adult |

## Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

## Objectives and benefits

**Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.**

**What's the aim of this project?**

Our aims are to gain a better understanding of endometriosis and to assess the efficacy of new treatments to prevent disease growth and recurrence.

**Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.**

**Why is it important to undertake this work?**

Endometriosis is a debilitating condition that affects an estimated 176 million women worldwide. In endometriosis, cells similar to the lining of the womb grow elsewhere in the body, typically in the abdomen around the ovaries and the bowel. These endometrial-like cells respond to female sex steroids with a chronic inflammatory reaction that is the leading cause of pelvic pain and infertility. Often dismissed as women's troubles, a lack of research and funding means sufferers can live in severe pain, unable to work or socialise. Current drug therapies carry side effects, are not suitable for patients seeking pregnancy and endometriosis often recurs upon discontinuation of drugs. There is a scientific and clinical need for a better understanding of endometriosis development/recurrence and the assessment of new treatments/delivery systems.

**What outputs do you think you will see at the end of this project?**

**New information**

It will increase our understanding of the disease. In particular, it will provide new information in relation to the local peritoneal environment (the abdominal area surrounding the lesions) in disease progression and following treatment with novel compounds. In this programme of work, we will also look to develop and investigate a model of recurrence and utilise this model to assess treatments.

Specifically, lesion size, hormone production, inflammation and metabolic pathways known to exacerbate the disease will be studied. This work should increase knowledge about the effect of new treatments and modalities on endometriosis development, progression and recurrence. It is anticipated that these treatments and delivery methods will reduce lesion formation and/or prevent their regrowth, thus providing important information with the aim to progress these compounds to the clinic for patients.

Information in relation to novel drug delivery platforms, including the suitability of hydrogels, will also be assessed. These modalities have yet to be studied as a local delivery system within the peritoneal cavity, where lesions are usually found. Surgery will be performed at similar times of the day to avoid body clock effects on inflammation, adhesion and local repair.

By monitoring the reproductive cycle via vaginal lavage and cytology, drug effects on reproductive function will also be determined. This measures the integrity of hormone signalling, that may indicate local verses systemic drug release. It will also show if contraceptive effects are reversible when treatments are discontinued.

**Publications**

Our research spans across many different scientific fields, including: endocrinology, pharmacology, pharmaceutics and material science. We plan to submit articles to high impact, open access journals, such as the British Journal of Pharmacology and the Journal of Controlled Release.

There is limited previous research on endometriotic lesion recurrence; therefore, this research is very novel. Publications will demonstrate validity of our model of recurrence as well as the effect of novel and/or localised treatments.

There is also a sparsity of hydrogel imaging research within the peritoneal/abdominal cavity, which would be of interest to drug delivery scientists studying diseases within that area of the body.

**Products**

If proof of concept is successful and lesion weight or endometriosis recurrence is significantly reduced, novel treatments and/or hydrogels could be considered as a possible, and viable, treatment option within the clinic.

**Who or what will benefit from these outputs, and how?**

**Scientific community**

Studying the local lesion environment with or without drug treatments would enhance knowledge about the cause of endometriosis and its development, progression and recurrence. This information would benefit other researchers within the same field as well as the general scientific community and general public.

**Translation for clinical benefits**

Successful placement and efficacy of the hydrogel-based delivery system could have clinical benefit for women with endometriosis. Introducing a localised drug delivery system as opposed to oral or injection-based treatments could reduce the severe side effects that often occur with systemic dosing, such as: weight gain, headaches, depression, reduced bone density and acne. Current intra-uterine devices carry risks of ejection from the body, infection and piercing of the uterus that would be mitigated by the hydrogel. By localising drug release, women receiving this treatment could have the option to conceive, which is not feasible with current hormonal therapies. Targeted delivery would therefore improve patient compliance, reproductive health and would also reduce the need for multiple surgeries. This would significantly cut healthcare costs, which are currently estimated at £8.2billion/annum in the UK alone, by saving the need for referrals, specialist centres, additional treatments and lost working time. The clinical benefit of these new medicines would be more long-term, beyond the lifespan of this project licence.

**How will you look to maximise the outputs of this work?**

We are collaborating with other research groups concerned with the peritoneal/abdominal cavity, regenerative medicine, imaging experts, gynaecologists and material engineers. This collaboration will allow for hydrogel formulation, live imaging and analysis of suitability for the clinic. This will provide

information on both drug release to endometriotic lesions as well as the prevention of adhesions, which commonly occur within this area of the body, particularly after surgery.

To avoid publication bias, we are committed to publishing all findings from this project, both positive and negative. Outputs will be maximised through publication of abstracts, papers, conference presentations, patient public involvement and public engagement activities.

**Species and numbers of animals expected to be used**

- Mice: 1000

# Predicted harms

**Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**

**Explain why you are using these types of animals and your choice of life stages.**

We require our chosen model to undergo an oestrous cycle, the equivalent of a human menstrual cycle. Hormonal changes, particularly in oestrogen, are essential for disease establishment and progression. We also wish to monitor if our chosen localised treatments disrupt this oestrous cycle. Therefore, it is necessary that the chosen animal is a sexually mature mammal. As a group, we have chosen to utilise mice.

**Typically, what will be done to an animal used in your project?**

There are two main categories of animals used within this project: donor mice and recipient mice.

**Donor Mice**

Donor mice will be humanely killed in order for their uterus to be removed and sectioned into small pieces. In order to analyse which phase of the oestrous cycle these mice are in, a vaginal wash will be conducted on these mice prior to being humanely killed.

**Recipient mice: disease establishment**

In all cases, recipient mice will undergo surgery under anaesthesia. The uterine fragments from the donor mice will be stitched onto the peritoneum of the recipient mice, where lesions commonly form in humans. Recipient mice will undergo a small incision of the skin and muscle layer in order to gain access to the cavity. Prior to surgery, these mice will be injected subcutaneously with opioid-based pain relief. The wound will be cleaned with betadine solution and their eyes will be treated with lacri-lube to prevent dryness/irritation. Recipient mice will then be split into key groups: systemic vehicle, systemic treatment, localised vehicle and localised treatment. Vehicle will be the same solution the chosen drug is suspended in. Mice that are treated systemically with treatment or vehicle will undergo repeated administration throughout the project. Depending on the drug type, this will be given either by injection or orally. Recipient mice given localised vehicle or treatment will not need these repeated doses as a

hydrogel will be administered during surgery. Some mice will have small volumes of blood taken to analyse drug exposure levels. A proportion of these animals will be humanely killed at specific time points to allow for extensive analysis of the lesions as well as the surrounding area.

**Recipient mice: established lesions and disease recurrence**

In order to analyse drug effects on established lesions or lesion recurrence, these recipient mice will undergo two surgeries. They will undergo the surgery as detailed above to establish endometriosis within the animal. These animals will then be allowed to recover for a period of 2 weeks. After this time, the animal will undergo a second surgery which is almost identical to the first. Some of these animals will have their established lesion removed, whilst others will not. Treatment will start at this stage, with the same groups still applying: systemic vehicle, systemic treatment, localised vehicle and localised treatment. Animals receiving localised vehicle or treatment will be given the hydrogel at the time of the second surgery. Animal receiving systemic treatment or vehicle will be given either repeated injections or oral doses. Some mice will have small volumes of blood taken throughout the experiment to analyse drug exposure levels. Vaginal wash will also be performed to determine drug effects on reproductive function. A proportion of these animals will be humanely killed at specific time points to allow for extensive analysis of lesion regression or recurrence.

**Live imaging**

Recipient mice from each of the two procedures described above may also undergo non-invasive, live fluorescent imaging. This will allow hydrogel localisation, lesion growth and recurrence to be monitored.

**What are the expected impacts and/or adverse effects for the animals during your project?**

The expected impacts of both protocols is the development of endometriotic lesions, which have been shown to establish within roughly 2 weeks after induction. As the model of endometriosis proposed within this project has been used and verified in the previous project licence, it has been shown to be well-tolerated by the animals, with no outwards sign of pain or distress.

Drugs previously tested within this animal model appear to also be well-tolerated by the animals with no visible adverse effects. Drugs that have yet to be tested within this model are well characterised within the literature.

Localised drug delivery vehicles being used within this project, namely hydrogels, are also well-characterised within the literature and appear to not have a toxic effect in numerous cell types/lines. This will be verified with our cells of interest within the laboratory prior to commencement of any animal work.

Daily vaginal wash was well-tolerated by the mice when performed for up to 4 weeks under our previous licence.

A potential impact of protocol 2 is aberrant wound healing due to second surgery. However, this will be assessed in a small pilot study to see potential adverse effects and their duration.

**Expected severity categories and the proportion of animals in each category, per species.**

**What are the expected severities and the proportion of animals in each category (per animal type)?**

Donor animals used (~20%) are humanely killed using Schedule 1 (mild).

Recipient mice (~80%), the maximum expected severity is moderate.

**What will happen to animals at the end of this project?**

- Killed

# Replacement

**State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.**

**Why do you need to use animals to achieve the aim of your project?**

Endometriosis is a complex disease with a relatively poorly understood cause. Past research has shown that the cause of endometriosis has a hormonal, immune and inflammatory element. Therefore, in order to correctly analyse the effects of localised treatment, an animal model is the only viable option as it takes into consideration all of these options. Work using cells or individual organs fail to account for all of these components.

**Which non-animal alternatives did you consider for use in this project?**

Prior to animal work, primary cells as well as cell lines have been utilised to monitor the effects of these drugs as a solution and within a hydrogel. Cells of interest and surrounding cell types were studied. Cell-based experiments on how to investigate cellular regrowth are also being investigated.

Studies investigating drug release from the hydrogels are being conducted within the laboratory, using media similar to the peritoneal/abdominal fluid.

**Why were they not suitable?**

Whilst methods involving primary cells and cell lines gave information on drug efficacy in cellular models, it does not provide detailed information on these drugs effects on interacting cell types and the complex environment surrounding the lesions. Laboratory methods used to monitor drug release only takes into account the diffusion of the drug. It fails to account for degradation of the hydrogel by enzymes and shear stress.

For this, we need a more complex, animal-based system to be able to monitor how these drugs are effective and can prevent disease progression/recurrence.

# Reduction

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

**How have you estimated the numbers of animals you will use?**

Working alongside a trained statistician, we utilised data acquired from previous animal work conducted within this group to calculate the minimum number of mice needed per treatment group and lesion number per mouse to find a biologically relevant reduction in lesion weight. Lesion weight was used as it is the most useful and clinically relevant measurement in the studies. This analysis allowed us to account for variation than can occur both between and within mice. It allowed a study with best chance of detecting meaningful outcomes to be designed.

**What steps did you take during the experimental design phase to reduce the number of animals being used in this project?**

We have utilised the NC3Rs experimental design tool in order to design our animal-based experiments. This allowed us choose appropriate groupings and blind the studies effectively. This design tool has also helped us identify any potential nuisance variables and how to avoid them. By doing this, we are reducing the likelihood of experimental replication, thereby reducing the number of animals used.

**What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?**

Donor uterus is segmented into many fragments (typically 12-15 pieces), therefore, fewer donor mice are required than recipient mice. Typically 3 lesions can be induced within a single recipient mouse. This reduces the numbers of recipient mice needed to see an effect of the drugs on lesions.

We have conducted pilot collaboration work to determine whether fluorescently-tagged hydrogels can be monitored within the peritoneum using live imaging. This pilot study investigated two different types of fluorescent tags. We humanely killed a small cohort of animals, from there, we conducted the study as planned in the protocol. This study confirmed that the hydrogel can be imaged within the mice, which means there is a reduced need for sacrifice at specific time points.

We will also conduct a small pilot study, which will involve analysing wound healing from dual surgery. This will provide information on the rate of wound healing and will ensure that dual surgery is feasible.

# Refinement

**Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare**

**costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.**

**Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.**

We will be using a mouse model to mimic endometriosis. This model requires both donor and recipient mice. Donor mice are humanely killed to allow for their uterus to be removed and segmented into small pieces. These segments are then stitched on to the peritoneum of recipient mice. This particular model will be used as it is one of the most well-established models of endometriosis within the literature. Previous work in our laboratory found that this method produced fluid-filled cysts that are characteristic of the disease. The cysts were also found to be responsive to hormones, which is also one of the main disease characteristics.

For the recipient mice, this technique requires a small incision on the ventral area, limiting pain and potential harm to the animal. From previous work, the animals recover well with no obvious adverse effects in relation to pain or distress and in the vast majority of animals we did not observe any changes in weight or normal behaviours.

Our previous work demonstrated that following surgery the lesions develop naturally without supplementing animals with hormonal treatment. We also conducted ovariectomies (removal of ovary function) in our original work to prove hormonal dependence of lesion development. These previous studies have allowed us to further refine our methodology and both steps have been removed from our studies.

**Why can’t you use animals that are less sentient?**

We require the mice for this project to be sexually mature mammals, as changes within the oestrous cycle are important to monitor and can affect lesion establishment, growth and recurrence. Therefore, using immature life stages or less sentient animals, i.e. non-mammals, is not scientifically appropriate.

**How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?**

Animals will be allowed to acclimatise to their environment to reduce any distress. Animals will also be trained to become accustomed to regular and appropriate handling.

For each step appropriate controls are in place to minimise any adverse effects for the animals and humane endpoints are clearly defined. Animals will be monitored by both the research group and the unit staff.

Animals will receive appropriate pain relief pre- and post-operatively. However, non-steroidal anti-inflammatory drugs will be avoided as the inflammatory response is involved in disease development.

Following surgery, animals will be allowed to recover from anaesthesia in a temperature controlled incubator. Surgical wounds on the animal will be subjected to after-care such as betadine solution to prevent infection. Lacri-lube will also be administered to each animal to prevent eye dryness and irritation whilst anaesthetised. Following recovery, animals will be closely monitored, weighed regularly and observed for any signs of distress or pain. We have also refined our animal handling techniques in line with current guidance.

**What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?**

We will follow the best practice guidelines highlighted within the document 'Responsibility in the use of animals in bioscience research' which is published by the NC3Rs.

We will also be following the best practice guidelines published by the NC3R concerning: blood sampling; grimace scale; how to pick up a mouse; housing and husbandry; procedures with care and rodent welfare hub.

We will comply with local practices and keep up to date with newsletters and bulletins sent by the animal unit.

**How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?**

We will regularly consult with our named information officer in order to stay knowledgeable in advances of the 3Rs. As well as this, we will keep up to date with any relevant literature on the 3Rs or published papers from the NC3R to ensure our work will always be within the recommended guidelines. We will also keep up to date with any news and bulletins provided by the animal unit in which we are undergoing our research.