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NON-TECHNICAL SUMMARY

Combined solutions for endometriosis and recurrent disease

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, Inflammation, Preclinical model, Fertility, Therapy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Adult, Pregnant 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?

To gain a better understanding of endometriosis and the associated complications caused by its growth and inflammation. We also seek to investigate the effectiveness of new medical solutions that prevent development and/ or return of the disease.

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 long term and often very painful condition that affects many women. It happens when tissue similar to the lining inside the womb grows in places it shouldn’t, such as on the ovaries, the pelvic area, or the bowel. This tissue responds to hormones just like the lining of the womb does, so it can thicken, break down, and bleed. But because it’s outside the womb, the blood has nowhere to go. This leads to inflammation, swelling, scarring, and ongoing pain. Some women also develop adhesions, which are bands of scar tissue that make organs stick together. These can cause even more pain and sometimes serious complications. Endometriosis can also cause extreme tiredness and difficulties getting pregnant.

Despite how common and disabling it is, endometriosis is often dismissed as simply “bad periods,” and research into the condition has been limited. As a result, many people suffer for years without proper diagnosis or effective treatment. This can affect every part of life including work, relationships, social activities and the ability to have children.

With around 190 million women worldwide affected, better treatments are urgently needed. Surgery to remove the abnormal tissue often only helps temporarily, and the symptoms frequently return. Current drug treatments can cause unwanted side effects, and many of them can’t be used by women who want to get pregnant. Symptoms often come back when the medication is stopped. Because of all this, there is a clear need for more research and better therapies so we can understand the condition fully and develop treatments that prevent endometriosis from developing, returning, or causing long-term complications.

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

New information

Our previous project allowed us to develop and validate a new model that mimics how endometriosis lesions return after treatment. We now aim to better understand what causes the disease, in particular, the inflammation, scarring and cyst formation that drive lesion growth and recurrence. This work will explore what happens in the peritoneal environment (the abdominal area where lesions form) and how it responds to new treatments and drug delivery systems.

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We will measure features such as lesion size, the thickness of any scar tissue, the growth of new blood vessels, levels of hormone, and naturally occurring substances involved in inflammation and healing. Based on these findings, and our previous research, we expect that the new treatments and delivery methods will reduce lesion development or prevent regrowth, providing evidence to help move these therapies toward clinical use and support fertility preservation.

We will also test new ways of delivering medicines, including gel-like materials (hydrogels) that can slowly release drugs, to see how safe, stable and effective they are. These formulations are being optimised for controlled drug release directly into the uterus or peritoneal cavity. To avoid variation due to the body clock, surgeries will be carried out at similar times of day.

By monitoring the reproductive cycle, we will determine whether treatments affect hormonal signalling or fertility and confirm that any contraceptive effects are reversible through follow up pregnancy outcomes.

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 benefit: improving outcomes for women with endometriosis

A hydrogel based drug delivery system could offer important clinical benefits for women with endometriosis. Because the drugs are released directly where they are needed, this approach could avoid the common side effects of tablets or injections, such as weight gain, headaches, mood changes, low bone density and acne. Unlike current intra uterine devices, hydrogels do not carry the same risks of being expelled, causing infection or piercing the uterus.

Local drug delivery may also allow women to try for pregnancy while receiving treatment, which is not possible with today’s hormonal therapies. This targeted approach could improve patient comfort, reduce the need for repeat surgeries and lower healthcare costs. Endometriosis currently costs the UK an estimated £8.2 billion each year through treatment, referrals, specialist care and lost work time.

Overall, these new treatments could offer longer lasting benefits for patients, although these may not be fully realised until after the project has finished.

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

We are collaborating with other research groups concerned with the peritoneal cavity, regenerative medicine, imaging experts, gynaecologists and material engineers. This will allow us to optimise formulations and assess hydrogel performance and safety. We will provide information on both drug release to endometriotic lesions as well as the prevention of complications such as adhesions, which commonly occur within this area of the body, particularly after surgery.

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All studies will follow the ARRIVE guidelines to improve the transparency, quality, reliability and reproducibility of our results. We aim to disseminate all findings from this work (subject to protection of intellectual property), including both positive and negative data. Outputs will be maximised through publication of abstracts, high impact, open access papers, conference presentations, patient public involvement and public engagement activities. Outputs will also help us to collaborate with other researchers and pharmaceutical/ clinical experts, supporting our move towards clinical trial adoption.

Species and numbers of animals expected to be used

- Mice: 650

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 are using mice because we require our chosen model to undergo a reproductive (oestrous) cycle, equivalent to a human menstrual cycle. Hormonal changes, particularly in oestrogen, are essential for disease establishment and progression. As a result, we decided to use post-pubertal young adult female mice (6 weeks and older). At this age, they have reached sexual maturity and are traditionally used in experimental models of endometriosis. Their short oestrous cycles (4-5 days) and gestation period (19-21 days) will help us to monitor whether our treatments disrupt normal ovarian function and/ or successful pregnancy, both of which would indicate potential adverse effects.

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

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

Donor Mice

We will check the stage of the female mouse’s oestrous cycle by gently washing the vaginal area with a small amount of fluid and looking at the cells under a microscope (a technique called vaginal lavage). This is a quick and minimally uncomfortable technique. When a donor mouse is at a stage where her natural oestrogen levels are high (typically in proestrus), she will be humanely killed and their uteri will be removed and sectioned into small pieces ready to implant into recipients. Using donor tissue taken at this natural high-oestrogen stage means we do not need to give the animals extra hormones, which is an improvement on older methods and helps reduce their overall burden.

Recipient mice: endometriosis-adhesion establishment

In all cases, recipient mice will undergo surgery and/or hydrogel placement under anaesthesia, which typically lasts <45 minutes. Mice receive opioid-based pain relief, their lower abdomen shaved, and lubricating eye ointment applied to prevent dryness/ irritation.

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To recreate the main features of endometriosis and the scar-like adhesions that can form with it, the mice will undergo a small abdominal operation. They recover well from this procedure. Tiny pieces of uterine tissue from donor mice are then gently stitched onto the inside of the abdominal wall of the recipient mice. This is the same area where endometriosis often develops in women. In some cases, the surface of the abdominal lining may be lightly scratched to encourage the formation of adhesions. This creates small areas of internal scar tissue (less than 1 cm^2), similar to what happens in patients. Within two weeks, the mice develop lesions, each smaller than the size of a pea, that closely resemble those seen in people with endometriosis. Some mice will then receive test treatments or control substances. These may be given through injections, by mouth, or applied directly to the affected area using gels placed inside the abdomen or uterus. In our previous work, these procedures and treatments were well tolerated and did not cause obvious adverse side effects.

Some mice will have very small amounts of blood taken so we can check drug exposure levels. This may cause brief, mild discomfort. We will again assess the stage of the female mouse's oestrous cycle by gently washing the vaginal area (a technique called vaginal lavage) to monitor how the treatments might affect the animals' normal reproductive cycle. At set time points during the study, some mice will be humanely killed so that we can carefully examine the endometriosis-like tissue and the surrounding area in detail. These studies usually last around 28 days, but in some cases may continue for up to 84 days so we can monitor how the disease and treatments change over time.

Recipient mice: endometriosis-adhesion recurrence

In a further set of mice, the effect of therapeutic compounds will be assessed on already established lesions and adhesions.

The mice will undergo surgery as detailed above to generate superficial endometriosis lesions and adhesions at specific sites on the lining of the abdomen. Two weeks post-surgery, a second surgery will be performed. During this, the existing adhesions will be examined and gently separated, and part of the endometriosis-like tissue will be removed, similar to what happens during surgery in patients. At this stage, the mice will receive test treatments or appropriate control substances. These may be applied directly to the affected area, for example using a gel, or given through repeated injections or by mouth for up to 28 days.

As well as checking reproductive function using vaginal cytology (the examination of cells collected by gentle vaginal cell sampling), blood samples may be taken at periods throughout the study to monitor hydrogel breakdown and determine drug concentrations in the circulating bloodstream. A proportion of these animals will be killed at specific time points to examine the degree of lesion regrowth and adhesion formation together with the effects on uterine and peritoneal environments. Recurrence studies will typically take a total of 42 days (potentially ranging up to 84 days to allow for observation after treatments).

Live imaging

Recipient mice from each of the two procedures described above may also undergo non-invasive, fluorescent imaging. This involves use of special dyes to track individual cells, organs (such as lesions), drugs, or hydrogels in real time inside the body without surgery or causing harm. This will allow us to safely monitor hydrogel localisation, lesion growth or recurrence and adhesion formation within the same animal over time.

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Pregnancy

A small number of healthy mice and mice that have received treatment may be bred in a controlled manner so that pregnancies can be closely monitored. We will assess fertility and pregnancy outcomes, including whether pregnancies occur, how long they last, the number of pups born, and their health and wellbeing. The study will end once these assessments have been completed.

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

During our project we will establish models of endometriosis and endometriosis-adhesions to mimic clinical disease using surgical techniques. The animals will experience some discomfort after surgery that may initially reduce their appetite but cause no lasting harm. Our last licence helped us to minimise laparotomy wound size to lower stress and aid recovery. The only outward adverse sign we observed was a brief reduction in mouse weight post-surgery (1-2 days) that then returned.

The drugs and hydrogel systems previously tested also appeared to be well-tolerated and daily vaginal lavage did not appear to cause any discomfort or stress. There is a risk that new drugs and local delivery devices could cause adverse side effects. To mitigate this, we will use repurposed and/ or well-characterised therapies using the route of least severity and the minimum number of doses to produce an anticipated beneficial effect.

Any change in weight and specific behaviours that would indicate pain or distress will be monitored and painkillers given if needed. Doses will be reduced and/ or treatments stopped if suffering is observed. At the end of the experiments, animals will be humanely killed and tissues will be taken for further laboratory tests.

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 mice (~20%) will be humanely killed (mild).

The maximum expected severity for recipient mice (~80%) is moderate.

What will happen to animals used in 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?

We regularly review the literature to incorporate non-animal alternatives into our research plans and use online tools, such as the Systematic Review Facility (SyRF), to review published data and meta-analysis of relevant pre-clinical studies. We have also utilised the Replacing Animals in Research Replacement Checklist in our searches. This helps us to incorporate What, Where, When, Who, How and Why questions to ensure a thorough exploration of opportunities to avoid animal use is routinely been performed.

We also study fluid and tissue samples donated by consenting women with and without endometriosis. We routinely use primary cell cultures (cells taken directly from human tissue and grown in the laboratory) to help guide and refine our animal work. These systems allow us to test how individual cell types respond to potential treatments. Some laboratory models can grow cells in simple three-dimensional structures, which gives a better approximation of living tissue than flat cell layers. However, we still cannot recreate the full complexity of endometriosis in non-animal systems, including complications like adhesions that form inside the abdominal cavity and these processes cannot be ethically tested directly in people. Because endometriosis involves hormonal, immune and inflammatory factors all acting together, an animal model is currently the only way to study how whole-body and local treatments behave in a dynamic, living environment that closely reflects the human condition.

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

Prior to animal work, human cells will be used to monitor the effect of potential therapies. These include primary endometrial and endometriosis cells as well as a mesothelial cell line (Met-5As) derived from the peritoneum. To support our 3Rs approach, our PhD student has started to develop a human endometriosis-peritoneal 3D culture model in collaboration with biomaterial colleagues at the facility.

We plan to look at how each ingredient in the hydrogel affects cells, and to check how well the drugs work and how safe they are for use in the body. We'll also use the system to study how cells grow back and how tissues produce fibres and collagen. In addition, we are characterising biomarkers and cells in peritoneal fluid from women with and without endometriosis and use a similar medium to assess drug release from the hydrogels. These studies should support / validate our in vivo findings.

Why were they not suitable?

Laboratory tests can show how drugs are released from a hydrogel and whether they affect individual cells linked to endometriosis; however, they cannot fully recreate what happens inside the body. For example, they do not account for how the body naturally breaks down the hydrogels or how movement of organs may affect drug release and retention at the treatment site. Animal studies are therefore needed to assess how well these new drug delivery systems work in real-life conditions and to determine whether they can help prevent the disease from progressing or returning.

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?

In conjunction with a statistics consultancy specialist, we used data acquired from our previous animal work to determine group sizes. This helped us to calculate the minimum number of mice needed per treatment group and lesion number per mouse to find a biologically relevant reduction in lesion size. Lesion volume was selected 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 and helped us to design our study with the best chance of detecting meaningful scientific outcomes. Calculations typically show that reliable results will be achieved using group sizes of 8.

For each drug study, we plan to randomly assign mice to one of four main treatment groups:

1) Vehicle controls (receiving the delivery substance without the active drug). This allows us to determine whether any observed effects are caused by the drug itself rather than the delivery substance.

2) Systemic drug treatment (receiving the active drug through whole-body administration). Dosing frequency will depend on how the drug is absorbed, distributed and processed by the body.

3) Local placement of hydrogel controls onto lesion sites or within the uterus (receiving the hydrogel carrier without the active drug),

4) Drug-loaded hydrogels onto lesion sites or within the uterus, enabling local drug delivery and release at the target tissue.

At the end of each study, we will score, collect and store as many tissues, fluids and cells as possible to maximise outputs. Where appropriate, treatment assignment will be blinded to the data analyst to ensure bias avoidance is adopted.

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

We have used the NC3Rs experimental design tool in order to design our animal-based experiments. This allowed us to 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; therefore, reducing the number of animals required. In addition the donor uterus is taken at proestrus when oestrogen levels are highest and the uterus is most vascular, thus reducing the number of donors required (1 donor to 4 recipients).

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

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The donor uterus is segmented into many fragments (typically 12-15 pieces); therefore, fewer donor mice are needed compared to recipients. Typically 3 lesions will be surgically-induced within a single recipient mouse, reducing the number of recipient mice needed to see drug effects on the lesions.

Pilot studies

In cases where the model has not been previously established by the group, e.g. inducing endometriosis-adhesions (no treatment) or new imaging-based techniques, the numbers will not exceed 6 per group. Characterisation of the abdominal environment has already been performed so no sham surgeries would be needed. Data from these initial pilot experiments will be used to define group sizes. Advice will also be sought from NC3Rs 'Guidance on Conducting a Pilot Study' and decision making will involve discussions with our collaborators.

We also carried out joint pilot studies using non-invasive imaging methods (e.g. fluorescent imaging and MRI) which let us look inside the body without surgery. These studies showed that the hydrogels stick well to the tissues they are applied to and stay in place for at least 21 days post-surgery in the abdominal cavity and 8–12 days in the uterus. They are then slowly broken down and removed naturally by the body. Because of this stability, using fluorescent markers within the tissues, drugs, or hydrogels may allow us to track what is happening inside the mice over time. This could reduce the number of animals that need to be killed at specific time points just to examine the tissues directly.

A small pilot study using our model of endometriosis recurrence also provided information on scarring and wound healing, that indicated feasibility of our approach.

At the end of the experiment, we will harvest as many tissues as possible at post-mortem. We will make the tissues we do not need available to other researchers if useful to them.

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 follow published guidelines to ensure maximum refinement of our models to mimic endometriosis and endometriosis recurrence. These require the donor mouse uterus to be removed, segmented and then sutured onto the peritoneum of recipient mice through a small incision site on the ventral area. We have refined the incision size for both first and second surgeries, limiting potential suffering to the animals. In our previous studies, the mice all recovered well (no loss in body condition score or change in normal behaviours).

This particular model of endometriosis is well-established within the literature. Previous published work from our laboratory confirmed that when donor tissues were taken in proestrus, lesions naturally

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developed into fluid-filled cysts that are characteristic of the disease in women. This has allowed us to further refine our methodology; steps to remove the ovaries and supplement with oestrogen have now been removed.

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

We require the mice for this project to be sexually mature mammals. Changes within the oestrous cycle and pregnancy 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. Moreover, studies comparing mouse and people indicate the potential for results to translate between the species.

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

Mice will be allowed to acclimatise to their environment to reduce any distress. They will also become accustomed to regular and appropriate handling, which is observed when we perform daily vaginal lavage. We have refined our animal handling techniques in line with current guidance.

We use a painkiller (e.g. buprenorphine) before a laparotomy so that it is active by the time the mouse recovers. An eye lubricant is applied to prevent dryness and irritation whilst under anaesthetic and the abdomen is cleaned with a topical antiseptic solution before and after surgery to lower risk of infection. The mice are then placed in a temperature controlled incubator whilst recovering from anaesthesia.

Following recovery, the mice will be housed in groups, weighed regularly, and closely monitored for signs of discomfort or ill-health. This will include monitoring their weight, appearance, breathing, posture, activity levels and facial expressions that may indicate pain or distress. If these are observed, the mice will be treated accordingly. Any that develop severe effects will be humanely killed.

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

We will follow the best practice guidance covered by ‘PREPARE: guidelines for planning animal research and testing’, 'Responsibility in the use of animals in bioscience research,' published by the NC3Rs and the recommendations of the Joint Working Group on Refinement (Morton et al., 2001).

We will also comply with the NC3Rs documents concerning: blood sampling, the grimace scale, how to pick up a mouse, housing and husbandry, procedures with care and the rodent welfare hub. It is also important for us to keep up to date with newsletters and bulletins sent by the internal facility communications team for local compliance.

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 / 3Rs manager and check the NC3Rs website to ensure our work stays up-to-date with the recommended guidelines. We regularly monitor

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subject-specific literature for scientific advances that may have a 3Rs impact.

We also subscribe to the NC3Rs, Understanding Animal Research and internal facility newsletters and communications as well as attend the Regional 3Rs symposia. We will also routinely use the Replacing Animals in Research Replacement Checklist in our searches. This helps us to incorporate What, Where, When, Who, How and Why questions to ensure a thorough exploration of opportunities to avoid animal use has been performed.

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