Study Title: FENOX – A Study into the Biology of Uterine Fibroids and Endometriosis at Oxford.

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Conflict of Interest Statement

The investigators declare no conflict of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

TABLE OF CONTENTS

1. SYNOPSIS

Study Title	FENOX – A study into the biology of uterine Fibroids and Endometriosis at Oxford.	
Internal ref. no. / short title	FENOX	
Study Design	Longitudinal observation and laboratory studies	
Study Participants	Women of reproductive age (18 years until menopause) who are planned to undergo surgery for endometriosis and/or fibroid-associated symptoms, or having unrelated gynaecological surgery.	
Planned Sample Size	1200 participants total (endometriosis and/or fibroids): Endometriosis: 500 participants who undergo surgery (including hysterectomy) for endometriosis, 100 participants without endometriosis having surgery for tubal sterilisation. Fibroids: 500 participants who undergo surgery for uterine fibroids or fibroid-associated symptoms such as abdominal pain, abnormal uterine bleeding, or fertility investigation, and 100 participants without uterine fibroids, who undergo hysterectomy for symptoms such as pain or abnormal uterine bleeding. In addition to this, we will make use of two existing tissue sample resources, i.e., fibroid and uterine tissue samples collected as excess tissue (Oxford Radcliffe Biobank, REC Ref 09/H0606/5+5), currently approximately 70 samples, and adipose tissue samples collected by the GTEx consortium from deceased organ donors (N = ~950).	
Planned Study Period	1st December 2017 – 30th September 2027 (recruitment ends September 30 2025)	
	Objectives	**Outcome Measures**
Primary	To identify the underlying mechanisms of endometriosis and uterine fibroids and their associated symptoms to improve the outcome of affected women.	We will use questionnaire data, medical records and sample analysis to investigate the genetic and molecular basis of the pathogenesis and symptoms of endometriosis and uterine fibroids.
Secondary	To identify novel biomarkers of endometriosis.	Prospective standardised questionnaires and samples will be collected according to EPHect (Endometriosis Phenome and Biobanking Harmonisation Project) standards. The correlation of data and endometriosis status will allow us to define novel biomarkers of the disease.
	To identify clinical subgroups of endometriosis and uterine fibroids.	Clinical notes and questionnaires in combination with sample data will be used to define clinical subgroups of patients.
	To understand the genetics underlying these conditions and explore the relevant downstream molecular pathways.	The molecular and genetic findings will be compared against control samples, public databases of disease-relevant molecular pathways, and *in vitro* experiments

		will be carried out to test hypothetical connections between the genetics and manifestation of disease.
	To investigate the relation between the presence of fibroids and the symptoms, *e.g*. abnormal uterine bleeding.	The blood vessels and the cells they are made of (endothelial cells) will be compared between tissue from women presenting with fibroids and those without.
	To identify novel drug targets.	The detailed comparison between tissue from women with fibroids and those without will yield differences in terms of proteins expressed; these can then be tested as targets using known or new drugs.
	To develop models of disease progression and prediction.	As data accumulate and genetic mechanisms become clear, hypotheses will be formed as to the likely progression of disease. These will be tested against the reports from the follow-up questionnaires
	To investigate conditions or symptoms associated with endometriosis and/or uterine fibroids, including: symptoms and characteristics of the female reproductive system (characteristics of menstrual bleeding, fertility, infertility, pregnancy outcomes), pelvic as well as non-pelvic pain conditions, metabolic phenotypes (polycystic ovarian syndrome (PCOS), obesity and fat distribution), cardiovascular conditions and symptoms, neuroangiogenesis and related neurological symptoms, immunological disorders, and cancers.	We will use questionnaire data, medical records and sample analysis to investigate the genetic and molecular basis of the pathogenesis and symptoms of conditions or symptoms associated with endometriosis and/or uterine fibroids, and to better understand the molecular signature of the relevant tissues in a healthy state.

2. ABBREVIATIONS

CI	Chief Investigator
CRF	Case Report Form
CTRG	Clinical Trials & Research Governance, University of Oxford
EPHect	Endometriosis Phenome and Biobanking Harmonisation Project
FENOX	Fibroids and Endometriosis Oxford
GCP	Good Clinical Practice
GP	General Practitioner
GTEx	Genotype-Tissue Expression project
HRA	Health Research Authority
ICF	Informed Consent Form
NHS	National Health Service
NRES	National Research Ethics Service
PI	Principal Investigator
PIS	Participant/ Patient Information Sheet
REC	Research Ethics Committee
TCRF	Trans-cervical resection of fibroids
WERF	World Endometriosis Research Foundation

3. BACKGROUND AND RATIONALE

Millions of women suffer from the consequences of endometriosis[1] and uterine fibroids[2,3]. These include pelvic and abdominal pain, abnormal uterine bleeding, infertility and miscarriages[4–7]. As such, these conditions not only affect women and their families in their everyday lives, but also have been shown to have an enormous socioeconomic impact for society in general: In the United States, fibroids are cited to be the cause for over 50% of hysterectomies[8], and direct costs for their treatment is estimated between 4 and 9 billion USD[9].

Clinically relevant, non-invasive diagnostic tests including biomarkers or imaging techniques do not exist for many forms of endometriosis[10–12] resulting in an average delay in diagnosis of 8-12 years. Current treatment options are associated with significant side effects and risks and include hormonal suppression/modification, surgical removal or, in the case of fibroids, embolization and MRI guided focussed ultrasound (MRgFUS).

Therefore, there exists a significant unmet clinical need to better understand the underlying mechanisms of these conditions and conditions associated with endometriosis and uterine fibroids, which will enable us to develop more specific diagnostic tests and will eventually lead to individualised treatment, with fewer side effects and better efficacy. To achieve this goal, it is essential to collect prospective high quality, standardised clinical and intra-operative data and corresponding biological samples. Our group has been at the forefront of the development of standard operating procedures and questionnaires for endometriosis as part of the World Endometriosis Research Foundation's (WERF) Endometriosis Phenome and Biobanking Harmonisation Project (EPHect)[13–16], and we are planning to establish similar standards in uterine fibroid research.

In the FENOX (Fibroids and Endometriosis in Oxford) study, we aim to improve our understanding of the underlying mechanisms of endometriosis and uterine fibroids and their associated symptoms by means of longitudinal observation and laboratory analyses. To achieve this, samples and clinical data will be collected from women undergoing surgery. These samples will be used in state-of-the-art biomedical assays (see section 7.5, 'Assays') to improve our understanding of the underlying biology of these symptoms in women with endometriosis and/or fibroids, which will lead to a better understanding of the conditions, stratification of patient groups and tailored therapies, and the development of novel drug targets and biomarkers for diagnosis and treatment.

Women attending clinics or admitted for surgical treatment will be asked to participate in this study. Once consented, they are asked to complete detailed questionnaires at baseline and then at intervals (6-8 weeks, 6 months, 1 year and 2 years) about their symptoms, medications, co-morbidities, and ethnicity. Consenting women will also be asked to donate biological samples such as blood, urine and saliva at baseline and optionally, some of these again at a follow-up visit after their surgery, in addition to tissue taken at time of surgery.

4. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary Objective To identify the underlying mechanisms of endometriosis and uterine fibroids and their associated symptoms to improve the outcome of affected women.	We will use questionnaire data, medical records and sample analysis to investigate the genetic and molecular basis of the pathogenesis and symptoms of endometriosis and uterine fibroids.	At the end of the recruiting period, i.e. from December 2025 onwards, the collected data and samples will be analysed and compared between endometriosis/fibroid cases, and non-affected controls.
Secondary objectives To identify novel biomarkers of endometriosis.	Prospective standardised questionnaires and samples will be collected according to EPHect (Endometriosis Phenome and Biobanking Harmonisation Project) standards. The correlation of data and endometriosis status will allow us to define novel biomarkers of the disease.	As above
To identify clinical subgroups of endometriosis and uterine fibroids.	Clinical notes and questionnaires in combination with sample data will be used to define clinical subgroups of patients.	At the end of the recruiting period, i.e. from December 2025 onwards, the collected data and samples will be analysed and compared between endometriosis/fibroid cases, and non-affected controls.
To understand the genetics underlying these conditions and explore the relevant downstream molecular pathways.	The molecular and genetic findings will be compared against control samples, public databases of disease-relevant molecular pathways, and *in vitro* experiments will be carried out to test hypothetical connections between the genetics and manifestation of disease.	As above

To investigate the relation between the presence of fibroids and the symptoms, *e.g*. abnormal uterine bleeding.	The blood vessels and the cells they are made of (endothelial cells) will be compared between tissue from women presenting with fibroids and those without.	As above
To identify novel drug targets.	The detailed comparison between tissue from women with fibroids and those without will yield differences in terms of proteins expressed; these can then be tested as targets using known or new drugs.	As above
To develop models of disease progression and prediction.	As data accumulate and genetic mechanisms become clear, hypotheses will be formed as to the likely progression of disease. These will be tested against the reports from the follow-up questionnaires.	As above
To investigate conditions or symptoms associated with endometriosis and/or uterine fibroids, including: symptoms and characteristics of the female reproductive system (characteristics of menstrual bleeding, fertility, infertility, pregnancy outcomes), pelvic as well as non-pelvic pain conditions, metabolic phenotypes (polycystic ovarian syndrome (PCOS), obesity and fat distribution), cardiovascular conditions and symptoms, neuroangiogenesis and related neurological symptoms, immunological disorders, and cancers.	We will use questionnaire data, medical records and sample analysis to investigate the genetic and molecular basis of the pathogenesis and symptoms of conditions or symptoms associated with endometriosis and/or uterine fibroids, and to better understand the molecular signature of the relevant tissues in a healthy state.	As above

5. STUDY DESIGN

SUMMARY

FENOX is a prospective study that aims to improve our understanding of the underlying mechanisms of endometriosis and uterine fibroids and their associated symptoms by means of longitudinal observation and laboratory analyses. Biological samples such as blood, saliva, urine, fat, peritoneal fluid and – if found - endometrial tissue or fibroids as well as detailed clinical and intraoperative data will be collected from women of reproductive age with and without endometriosis- and fibroid-associated symptoms, such as pain, abnormal uterine bleeding and infertility. Women undergoing surgery for these conditions, and women undergoing surgery for unrelated gynaecological conditions as part of their normal clinical management will be asked to participate. All women attending clinics receive a letter informing them of ongoing research. For face-to-face recruitment, eligible women will be identified initially by research nurses or clinical staff during clinic visits. Once a woman has expressed an interest in participating in this study, they will be consented by a member of the research team. For remote recruitment, eligible women will be initially identified via pre-op, surgical and clinic lists. The contact details of eligible women will be located on EPR and they will be approached by the research team about the study by phone, text or email. If an eligible woman expresses an interest in participating in the study, a member of the research team will receive remote consent. Alternatively, a woman approached remotely about the study may prefer to give face-to-face written consent to the study team on the morning of her surgery. (Nb. It is not feasible to both approach and consent a woman to the study on her surgery day due to time constraints.).

Blood, saliva and urine will be taken prior to surgery. Tissue and peritoneal fluid (where applicable) will be taken at the time of the scheduled surgery.

In order to determine the effect of the surgical removal of the fibroids on the local tissue, it is necessary to take an additional endometrial biopsy after the planned surgical intervention. This sample will be timed to synch with the same time point in the menstrual cycle that the original sample was taken, and thus will give us a unique insight into the biology of the conditions. During this visit, blood and urine samples will be taken again also. Women can opt in or out of the additional clinic visit where these samples would be taken.

Women will be asked to complete questionnaires appropriate to their condition on paper, online or into their electronic handheld devices (health, pain, medication and, initially, ethnicity) at different time points. There will be a lengthy questionnaire at baseline and then shorter versions post-operatively at 6-8 weeks, 6 months, 1 year and 2 years after surgical intervention.

SAMPLES

- Blood samples (up to 50 ml, venepuncture), urine (micturition) and saliva (spit) will be taken prior to surgery.
- During surgery, tissue samples will be taken as specified in section 7.3 below.
- In women opting in, an additional endometrial biopsy will be taken during a follow-up visit at least three months after surgery. This can be done in an outpatient setting, and the taking of an endometrial biopsy in this setting using an endometrial sampling device (*e.g.* pipelle or curette) is an established technique. A blood and urine sample will be taken again also.

6. PARTICIPANT IDENTIFICATION

6.1. Study Participants

Across both disease arms (endometriosis *and* fibroids), we plan to recruit up to 1200 women of reproductive age (18 years until menopause) who are planned to undergo surgery. 500 of these will have endometriosis, and 100 participants without endometriosis having surgery for tubal sterilisation or for other reasons and act as controls. A further 500 participants who undergo surgery for uterine fibroids or fibroid-associated symptoms such as abdominal pain, abnormal uterine bleeding, or fertility investigation, and 100 participants without uterine fibroids, who undergo hysterectomy for symptoms such as pain or abnormal uterine bleeding.

In addition to the 1200 newly recruited participants mentioned above, we will make use of existing tissue sample resources, including fibroid and uterine tissue samples collected as excess tissue (Oxford Radcliffe Biobank, REC Ref 09/H0606/5+5), currently approximately 70 samples, as well as visceral and subcutaneous adipose tissue samples collected from deceased organ donors by the GTEx consortium to which we have obtained access (N = ~950). The protocol of the GTEx project, which is a large collaboration between several academic institutions in the United States, was approved by Chesapeake Research Review Inc., Roswell Park Cancer Institute's Office of Research Subject Protection and the institutional review board of the University of Pennsylvania. Recruitment centres also obtained independent approval from their local institutional review boards. A material transfer agreement between the University of Oxford and the Broad Institute in Boston (US), where the samples from the GTEx consortium are currently stored, has been signed.

6.2. Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study.
- Female, aged 18 years or above (before menopause).
- Women undergoing planned surgery (including hysterectomy) for endometriosis- and/or fibroid associated symptoms such as abdominal pain, abnormal uterine bleeding, or for unrelated gynaecological conditions (*e.g.* fertility investigation or for laparoscopic tubal sterilisation).

6.3. Exclusion Criteria

The participant may not enter the study if **ANY** of the following apply:

- Women who are pregnant.
- Women who are unable to read, or to understand written or spoken English.
  History of cancer/ diagnosis of current cancer.

7. STUDY PROCEDURES

7.1. Recruitment

After general information through a generic letter with information about ongoing research which every patient will receive prior to her outpatient appoint, for face-to-face recruitment, eligible women will be identified initially by the research nurses or clinical team during clinic visits. The study research nurses will then contact those women interested in participating in the study. For remote recruitment, eligible women

will be initially identified via pre-op, surgical and clinic lists. The contact details of eligible women will be located on EPR and they will be approached by the research team about the study by phone, text or email.

7.2. Screening and Eligibility Assessment

Women attending clinic appointments for endometriosis- and fibroid-associated symptoms such as pain, abnormal uterine bleeding, and infertility will be asked to participate by clinical staff or by the authorised study research nurses. Women undergoing surgery for these conditions, and women undergoing surgery as part of their normal clinical management (*e.g.* laparoscopic tubal ligation or hysterectomy; they would be the control patients) are eligible to participate in the study. Due to the COVID-19 pandemic, some appointments for eligible women are virtual, necessitating a supplementary remote recruitment pathway into the study. Eligible women will be initially identified via surgical, pre-op and clinic lists by authorised study staff.

7.3. Informed Consent

For face-to-face recruitment, prior to giving consent, and usually during their pre-operative assessment visit, women will be given the relevant patient information sheet and consent form to read. Written consent will be received by a trained member of the research team.

Written versions, with verbal explanations, of the patient information sheet and the consent forms will be presented to the participants detailing the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study.

Consent will be obtained by a suitably qualified and experienced member of the study research nurse team and will have been authorised to do so by the Chief Investigator. Consent will be obtained either verbally over the phone by a study research nurse or in person on the day of surgery via a signed paper or electronic consent form with the participant's dated signature and the dated signature of the person who presented and obtained the informed consent recorded. When consent is obtained in person and electronically, the participant will be asked to type their name, confirm their entry and the date, rather than sign, at the end of the electronic consent form.

A copy of the signed informed consent and the patient information sheet will be given to the participant or emailed if taken electronically. The original signed form will be retained at the study site, with electronic copies stored in a secure, encrypted and regularly backed up network drive.

For remote recruitment, a letter, text or email containing an invitation to participate will be sent to women identified as eligible for the study from clinic, pre-op or surgical lists. The mode of communication will depend upon the contact details the woman has supplied on her Electronic Patient Record (EPR). The preferred method of primary contact will be email. If contacted by email or letter, a copy of the verbal consent form and the patient PIS will be attached. These primary communications will give brief

information about the study, inviting the recipient to respond if they have immediate questions, and stating that they will be phoned by the study team with regard to study in either a) five days if contacted by letter b) 48 hours if contacted by email or text. Recipients will also be told that if they do not wish to be further contacted then they should communicate this to the study team by phone, text or email.

After the set time period has passed, potential participants (who have not expressed they do not wish to be contacted again) will be called by the research nurses who will explain more about the study. The maximum number of times a potential participant will be called is twice. When a call is not picked up by a woman, the team may leave an answerphone message to state their purpose in calling and give their contact details should the woman wish to either a) find out more about the study or b) ask not to be contacted again. If no contact is made after two calls, the research team will interpret this as the woman declining participation in the study.

When telephone contact is made with a woman, if she does not yet have the PIS/verbal ICF, a method of getting them to her will be sought. Once the woman has the documents and has had time to read them, the PIS will be discussed and any questions answered by the research team. Should the woman wish to participate she can choose to either a) be read the statements on the verbal ICF by a research nurse who will then document the woman's consent or b) complete a written consent form face-to-face with a research nurse on the day of her surgery (NB. It is not possible both to approach and consent women to the study on the day of their surgery due to time constraints.).

When consent is obtained verbally, a study research nurse will call interested participants who have not opted out and offer to answer any questions the interested patient may have. The nurse will read through the verbal consent form and request affirmative verbal consent for each point. The consent form will be filled in by a research nurse whilst on the phone with the interested participant: providing a yes/no response for each point, the name of the participant, the name of the nurse taking verbal consent, the date, and adding an electronic signature. A password will be generated by the study research nurse using KeePass, a Medical Science Division approved password manager. Each participant will be given a unique study ID and the generated password will be stored in KeePass and only identifiable via this ID. The KeePass database is encrypted using the most secure encryption algorithms currently known (AES-256, ChaCha20 and Twofish) and is only accessible using SSO login credentials. The completed consent form will be saved as a password protected PDF on the University of Oxford's High Compliance System, a high security service provided by MSD-IT that is only available onsite using hardwired University network approved devices, with limited authorised access. The recruited patient will be emailed a copy of the password protected PDF via the NHSmail @nhs.net email service, a secure service conforming to DCB1596 email standards. The email will be encrypted using the [SECURE] subject keyword flag. The minimum identifiable patient data will be included in emails (*e.g*. NHS number only, not name, address, DOB etc). The recruited patient will then be sent a second email containing the password to unlock their consent form via the NHSmail @nhs.net email service. Consent will be documented in the patient's electronic notes.

The consent form for this study allows for the participant declining consent for any procedure that she is not comfortable with, while remaining eligible as a participant of the study. For example, if a participant did not want a uterine biopsy used in the study, she would not initial the corresponding box on the consent form and insert 'No' instead. When consent is obtained electronically, there will be 'Yes' and 'No' buttons for each question and, again, the participant will be asked to type their name, confirm their entry and the date, rather than sign, at the end of the electronic consent form.

7.4. Baseline Assessments

Consented participants will be asked by the research team to complete a baseline questionnaire before their surgery as appropriate to their condition.

This may be given to them in paper format during a clinic visit, sent to them in the post, or e-mailed before surgery.

When completing the questionnaires electronically, participants will be provided with a unique access code and can fill in the questionnaire via a browser on any electronic device, including their personal hand-held device. Encrypted Apple iPads are available for participants to fill in questionnaires whilst at the Hospital.

Questionnaire data provided before consent is provided will be withheld from the research team until written informed consent is obtained, and destroyed if this is not granted.

All participants will be sent further questionnaires at different time points (approximately 6-8 weeks, 6 months, one year and two years after surgery). Participants may be reminded (three times by the contact method(s) chosen by the patient when consented) to return completed questionnaires via mail, email, phone or text or similar. Patients may also be sent periodic study updates in the form of a study newsletter if the patient agreed to future contact when consented.

On the day of surgery, they will be asked to provide a mid-stream urine and a saliva sample. In addition, blood will be collected by peripheral venepuncture. The procedures will be explained to the women again and they will be given the opportunity to ask questions. Assessment of the presence and extent of disease will be performed by the operating surgeon.

Samples that may be taken at time of surgical procedures are as follows:

Laparoscopy for suspected endometriosis:

> Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue if present, peritoneal biopsy.

Laparoscopy for uterine fibroids:

> Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue (if present), peritoneal biopsy, fibroid tissue, myometrial biopsy.

Laparotomy for uterine fibroids:

> Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue if present, peritoneal biopsy, fibroid tissue, myometrial biopsy.

Trans-cervical resection of uterine fibroids (TCRF):

> Endometrial biopsy, fibroid tissue, myometrial biopsy

Laparoscopy for tubal sterilisation (Controls):

Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, peritoneal biopsy, myometrial biopsy

Hysterectomy:

Whole uterus as excess tissue from women with fibroids as well as from controls without fibroids, who undergo surgery for other indications (such as heavy menstrual bleeding, or pain), peritoneal fluid aspiration, fat tissue biopsy and peritoneal biopsy.

Women undergoing hysterectomy for benign causes such as abnormal uterine bleeding or pain will be asked to donate part of their uterus for research. Hysterectomy specimens are excess tissue and would be discarded otherwise.

In addition, during surgery, the surgeon will record digital photographs of the inside of the abdomen and/or uterine cavity as part of routine clinical care, which will also be stored on a secure server identified by the participant's study ID. Intraoperative findings will be recorded by the surgeon and anonymised data collected.

7.5. Subsequent Visits

All included women with a uterus will be asked to contact the study team when the next menstrual period after the procedure started, in order to calculate the length of that cycle. This is important in order to account for the changes that occur in the uterus during the menstrual cycle, and to enable us to distinguish between the effects of the cycle and the disease.

One subsequent visit will be made by participants who consent to this, dependant on research capacity. They will have another endometrial sample taken at least three months after the surgical intervention in an outpatient setting. The taking of an endometrial biopsy in this setting using an endometrial sampling device (*e.g*. pipelle or curette) is an established technique, takes approximately 30 minutes, and there is only a minimal risk of bleeding. In addition, blood and urine samples will be taken also. Pregnant women will not be eligible for the subsequent visits.

All women will be contacted by a member of the medical or study team and asked to fill in further questionnaires at different follow-up time points (approximately 6-8 weeks, 6 months, one year and two years). Reminders will be sent three times by the contact method(s) chosen by the patient when consented. Women will also be asked if they can be contacted in the future for any further studies approved by an ethics committee.

Women will be able to subscribe to a research newsletter. This will ensure a closer connection to the research and clinical team and has been very popular with participants in other studies.

7.6. Sample Handling

Samples will be obtained by the interventions listed below (section 8). Only the study team will have access. Biological samples will be stored at -20°C or at -80°C for use in current and future studies until exhausted. Disease and control samples will be stored under the same conditions. For the purposes of this study, and if participants agree, samples may be analysed at Oxford, or they may be transferred to a third party/study collaborator, including industrial partners, for analysis at their facility. If participants agree, samples will be moved to a Research Tissue Bank after the completion of their analysis for the study. Samples remaining at the end of the study may be moved to a Research Tissue Bank, or stored and used in future ethically approved studies. Samples will be made available in anonymised form.

To investigate the relationship between uterine fibroids and symptoms such as abnormal uterine bleeding and pain, we aim to collect endometrial and myometrial samples alongside the fibroids themselves, to be able to detect the effects of fibroids on their surroundings.

We also intend to use the endometrium and endometriotic samples, one of the blood samples and fat samples to look for genetic factors and molecular pathways that can lead to endometriosis or uterine fibroids. Additionally, we will use control samples to better understand the cell type-specific molecular signature of these tissues in a healthy state, which will provide us with a reference against which we can assess the pathogenic pathways of uterine fibroids and endometriosis. The samples for this analysis will also be anonymised so that we do not know specifically which patient they came from. However, all samples are identifiable with printed label and location detail, participant ID, sample type and colour-coded cap.

Blood:

50 ml approx. Samples are centrifuged for 10 minutes at 2500g at 4°C. These are divided into: EDTA plasma (lavender top tubes): 5x 125ul, 5x 225ul, 2x 1ml, 1x 2ml; 3x buffy coat; 2x 2ml erythrocytes. Silica serum (red top tubes): 5x 225ul and 1x remaining serum (6x aliquots). SST serum (gold top tubes): 5x 225ul and 1x remaining serum (6x aliquots). The different vials are colour-coded and frozen at -80°C.

Urine:

20 ml. One aliquot of 5 ml is frozen directly at -80°C; the remaining is centrifuged at 1000- 3000g at 4°C for 5 minutes to create 5 aliquots 2ml cell-free supernatant also frozen at -80°C.

Saliva:

A spit sample of approximately 1ml is taken on ice. One aliquot of 200 µl is frozen at -80°C, the rest is centrifuged at 1000g for 2 minutes at 4°C, 2 x 200 µl of cell-free supernatant are frozen at -80°C. One aliquot of 50µl of cell-free supernatant is combined with 200 µl of RNA-preserving buffer (RNA later, Qiagen, Germany) and then frozen at -80°C.

Faeces:

Whole stool samples will be collected. Samples will be homogenized, flash frozen, and stored at -80°C for further analysis. Patients may be provided with a stool collection and preservation tube during pre-surgery clinic visits or a tube will be posted along with study literature and patients will

be asked to collect their own stool sample which will then be returned to the research nurses on the day of surgery.

Peritoneal fluid:

During surgery, the peritoneal fluid will be collected on ice. Depending on the volume (up to 20 ml), samples are centrifuged for 5 minutes at 900g at 4°C, creating x1 1ml aliquot and approximately 5 2ml aliquots of cell-free supernatant created will be stored at -80°C. The pellet (cells) will also be stored at -80°C for further analysis.

Endometrium (*e.g*. pipelle or curette), endometrial lesions (peritoneum), abdominal fat, myometrium, fibroid tissue:

All tissue will be collected on ice and divided for storage at -80°C and at room temperature. An endometrium sample will also be fixed in paraformaldehyde and ethanol for menstrual phasing. Parts of fresh tissues will be used for culturing experiments, in order to test compounds, drugs or similar agents on primary cells.

Vaginal swab:

A vaginal swab will be performed during surgery or clinic visit or a self-administered swab will be provided to patients during pre-surgery clinic visits or posted along with study literature and returned to the research nurses on the day of surgery. Swab specimens will be stored at -20°C to -70°C for further analysis.

Hysterectomy:

In agreement with the local pathologist, whole uteri will be taken on ice and used for perfusion experiments within 24 hours before being transferred to pathology. Tissue samples of myometrium, endometrium, fibroid and fibroid-associated vasculature (if present) will be taken and stored at -80°C and – after fixing in paraformaldehyde and ethanol – at room temperature as the other tissue samples above.

Assays

RNA analysis:

RNA from each sample will be isolated by standard methods. Gene expression studies will be carried out between cases and controls (*e.g*. endometriosis vs non-endometriosis patients, or fibroid bearing women vs women without fibroids) using quantitative real-time PCR assays, whole RNA sequencing methods, RNA microarrays and spatial transcriptomics.

Protein analysis:

Proteins will be extracted from tissue samples using standard methods. The expression and amount of proteins will be analysed by immunoblotting for specific proteins of interest, and by proteomics methods using the MALDI/SELDI platform. Tissue sections will be used to detect the expression of markers of interest *in situ*, using standard immunohistochemistry and spatial proteomics.

Cells:

Fresh tissue will be dissociated into single cell suspensions. From these, the diverse cell types (*e.g*. endothelial cells) will be grown in incubators *in vitro* in order to study differences in cell behaviour between cases and controls, and to test compounds and drugs. Cells will be analysed by microscopy, flow cytometry and immunocytochemistry methods.

Similarly, cells isolated from peritoneal fluid or blood will be analysed using these methods.

Secretome analysis (perfusion):

Whole uteri with and without fibroids will be perfused with a suitable buffer (Krebs-Henseleit, or optimised versions of this) for up to 8 hours. The perfusate will be analysed by proteomics methods (see above) to detect factors secreted by the fibroids.

Microscopy:

Tissue blocks (up to 5 cubic millimetres in size) from perfused uteri will be stained with antibodies against markers of blood vessels and fibroids, and leakiness, and be recorded in a confocal microscope in order to render a three-dimensional image of the blood vessels *in situ*. The detailed study of these will allow us to determine whether there is a significant difference in the architecture of blood vessels in uteri with fibroids compared to those from uteri without fibroids.

7.7. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively, having been missed at screening)
- Withdrawal of Consent
- Loss to follow up
- Loss of mental capacity

Withdrawal from the study: At the point the participant withdraws from the study, we will ask for consent to retain samples and data collected up to that point.

Withdrawn participants will not be replaced.

The reason for withdrawal will be recorded in the CRF.

7.8. Definition of End of Study

The end of study is six months after the locking of the study database, to allow for completion of data analysis.

8. INTERVENTIONS

Non-clinical Interventions

Questionnaires:

As appropriate for their study arm – endometriosis or uterine fibroids – participants will complete specific questionnaires about general health, pain sensitivity, medication and menstrual history before their surgery. Additionally, those women with endometriosis will be asked to complete the Endometriosis Health Profile (EHP-30) Questionnaire, while the questionnaire for women with fibroids contains a section on their quality of life (UFS-QoL[17]). Clinical questionnaires are completed by participants on paper or digitally via REDCap (REsearch Data Capture), a secure web application for building and managing online surveys and databases approved and supported by Oxford Univeristy, with digital data stored securely on University servers. The follow-up questionnaires ask about symptoms and changes in menstrual history as relevant. The control groups would be given the same questionnaires as the women with the respective condition, and asked to omit questions that are not applicable to them.

Medical records:

We will obtain clinical data (menstrual cycle phase, medication, pain and menstrual bleeding status, photos from surgery) from the patients' medical records.

Clinical Interventions

Venepuncture:

Taken by an appropriately trained member of the clinical or research staff.

Collection of other bodily fluid sample:

Urine and saliva samples donated by the patient and sample prepared and analysed by a member of the investigative team.

Tissue collection

Tissue/fluid (*e.g*. fibroids if present) will be collected as part of routine surgical management apart from:

Laparoscopy:

Peritoneal fluid will be aspirated, biopsies from endometrium (*e.g*. pipelle or curette), abdominal fat tissue, myometrium, and peritoneum (excision) will be taken during surgery.

Additional Risk: Minor bleeding, uterine perforation (<1%), additional length of surgery by 5 min.

Myomectomy/hysterectomy:

Endometrial, myometrial and/or fibroid tissue biopsies will be taken during surgery. Hysterectomy samples will be used in structural analysis assays *ex vivo* in close discussion with the clinical pathologists.

Additional Risk: Minor bleeding, uterine perforation (<1%), additional length of surgery by 5 min.

Trans-cervical resection of fibroids:

endometrial and myometrial biopsies will be taken during surgery.

Additional Risk: Minor bleeding, uterine perforation (<1%), additional length of surgery by 5 min.

Additional endometrial biopsy:

Additionally: In women opting in, an additional endometrial biopsy will be taken during a follow-up visit n an outpatient setting. The biopsy of the endometrium is a simple, routine procedure and takes about 30 minutes.

Additional risk: Minor bleeding, uterine perforation (<1%), short period of discomfort.

Women will be asked to consent to the use of samples and clinical data collected as part of this research and in future research. Women, if they consent, will potentially be contacted for future studies approved by an ethics committee.

9. SAFETY REPORTING

For this study, it is conceivable that additional procedures may result in bleeding. However, if this resulted in a scenario mentioned below, it would constitute an SAE and needed to be reported to the sponsor.

9.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect

Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

9.2. Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related'

(resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form (see HRA website).

10. STATISTICS AND ANALYSIS

10.1. Description of Statistical Methods

The necessary methods will depend on the analysis undertaken. As this is a prospective sample and data collection study, there is no randomisation of patients as all women will undergo surgery as part of their routine clinical management. We routinely use SPSS, Graph Pad Prism, STATA, Python and R for analysis, and employ T-tests, ANOVA, correlation coefficient analysis and similar methods. Due to the exploratory nature of this study, various additional statistical techniques may also be used to fully explore the relationships in the data but all methods will be fully documented.

10.2. The Number of Participants

It is now recognised that both endometriosis and uterine fibroids are very heterogeneous conditions. Our previous studies[18,19] and systematic reviews[20,21] have clearly identified a lack of sufficiently powered studies. Multiple large-scale research collaborations are currently in place investigating different aspects of endometriosis[22], and we plan similar efforts for uterine fibroids. Therefore, large patient numbers are needed.

The Endometriosis CaRe Centre at Oxford is the UK's largest endometriosis centre. Similarly, as a tertiary referral centre, we see many women with fibroid–associated symptoms. As a result, we have the unique opportunity to collect large amounts of data and samples, which is essential to produce clinically meaningful outputs. Given our current recruitment rate (endometriosis: 100/year, uterine fibroids, 200/year) we estimate the recruitment of approximately 1200 women over the course of the study (500 endometriosis patients + 100 non-endometriotic controls, 500 fibroid patients + 100 non-fibroid controls).

In addition to the 1200 participants recruited as described above, we will make use of two previously collected sets of tissue samples. Fibroids collected as excess tissue under the Oxford Radcliffe Biobank (ORB, REC Ref 09/H0606/5+5) will be included in this study, currently approximately 70 samples. We will also use visceral and subcutaneous adipose tissue samples collected from deceased organ donors by the GTEx consortium (N = ~950) to which we have obtained access. The GTEx study protocol was previously approved by all relevant institutional review boards.

10.3. Analysis of Outcome Measures

All samples excluding those from patients who withdraw consent will be included in the analysis of outcome measures.

Laboratory data will be analysed using assay-specific software packages employing univariate and multivariate pattern recognition methods (*e.g*. principal component analysis, partial least squares, stochastic neighbour embedding algorithms) between sample groups. Correlation with questionnaire data

will allow us to validate prospective markers of disease. In addition, we will use laboratory data to predict disease severity (revised American Fertility Score), quality of life (EHP-30), pain measures and improvement of symptoms as per follow-up questionnaires. For the multivariate predictive methods, a test set of approximately 30% of each treatment group will be selected at random. This may be selected in a stratified method and exclude patients that have particularly extreme values. Patients not included in the test set will make up the training set. Models will then be built on the training set and assessed for predictability on the test set.

The analysis will be performed on the whole data set. However, if some influential differences are seen, then the women with endometriosis will be matched to corresponding women without endometriosis, or women with fibroids to women without fibroids, and the analysis based on these matched pairs.

11. DATA MANAGEMENT

11.1. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

11.2. Data Recording and Record Keeping

Each participant will receive a unique study number, which will then be used throughout the study. A study master sheet linking patient identifiable data (name, DOB, hospital and NHS numbers) with the unique study number will be stored in a password protected file separate from all other main study files on the University of Oxford's High Compliance System, a high security service provided by MSD-IT that is only available onsite using hardwired University network approved devices, with limited authorised access and in a file separate from the main study file. Hard copy study documents will be kept in a locked room at each participating centre. Research data will therefore be using non-identifiable data, and all records will be identified only by this study number. All other study data will be stored on encrypted and regularly backed-up Oxford University servers, with access limited and restricted by SSO certification. The participants will only be identified by study number in in all study datasets. The name and any other identifying participant details will **NOT** be included in any electronic file distributed to researchers and collaborators.

Where participants consent, coded genetic data and limited relevant details including, age, gender, information about body habitus, biochemistry etc. can also be made available to collaborators and to the National Institute for Health Research (NIHR) Bioresource (http://bioresource.nihr.ac.uk/), a panel of thousands of volunteers, who are willing to be approached to participate in research studies investigating the links between genes, the environment, health and disease.

12. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1. Declaration of Helsinki

This study will be conducted in accordance with the principles of the Declaration of Helsinki.

13.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

13.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

13.4. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

13.5. Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all study documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the UK General Data Protection Regulation (UK GDPR) and Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

13.6. Expenses and Benefits

There will be no payments made to study participants.

13.7. Other Ethical Considerations

Participants unable to consent for themselves will not be included in the study.

Patients under clinical management for infertility will be approached in a most sensitive manner by our experienced and well-trained team.

It is unlikely that our genetic analysis of the participants will reveal anything relevant beyond their normal clinical care so we do not plan to report any such findings to them or their GPs.

14. FINANCE AND INSURANCE

14.1. Funding

Funding for this study has been obtained from the Nuffield Department of Women's and Reproductive Health under the Oxford/Bayer-Alliance for Women's Health. Additional funding for the analysis of tissues obtained from the GTEx tissue biobank will be provided by Prof. Cecilia Lindgren's Wellcome Trust Investigator Award, 'The molecular etiology of central obesity: moving from variant to function using large-scale data-driven approaches'.

14.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

15. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the Nuffield Department of Obstetrics and Gynaecology under the Oxford/Bayer-Alliance for Women's Health, and Professor Cecilia Lindgren's Wellcome Trust investigator award. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

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17. APPENDIX B: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1 (SA1 – A002)	2.0	28th February 2019	Thomas Tapmeier	Removed the adoption of ENDOX samples from the protocol. Added related conditions to the list of secondary objectives. Changed addresses and e-mail addresses according to the new name of the department. Added the option of completing questionnaires online.
2 (MA-1 – A004)	2.0	26th March 2020	Tabitha Wishlade	Remote recruitment pathway added to study
3 (SA2 – A005)	3.0	23rd October 2020	Kurtis Garbutt	The protocol and PIS have been modified to allow for the remote recruitment pathway. Further, a remote recruitment letter/email and text message have been introduced to aid recruiting in this fashion. A verbal ICF has been created to allow the research team to receive consent remotely. Finally, the generic gynae letter has been amended as recruitment to studies will commence despite the pandemic. It also now informs women that remote recruitment is a possibility.
4 (SA3 – A006)	4	17th December 2020	Kurtis Garbutt	Reduction in overall recruitment numbers. Change to the overall endpoint of the project via the removal of a 5-year follow-up survey, thus reducing the project

				endpoint from 2028 to 2025. Digital questionnaire collection outlined. Additional collection of vaginal swabs and changes to sample collection (volume, aliquot number). Minor changes to project documents, including updates to the study protocol, consent, PIS and participant correspondence to include proposed procedure changes and better outline previously approved changes. Addition of reduced surveys for hysterectomy patients to avoid any potential emotional impact.
5 (SA4 – A007)	5	30th March 2021	Laura Wittemans, Kurtis Garbutt	Inclusion of adipose tissue samples which were previously collected from deceased organ donors by the GTEx consortium. Inclusion of additional sources of funding for the data generation on these adipose tissue samples. Addition of an additional co-investigator, i.e., professor Cecilia Lindgren. Minor change to the assays which we will deploy to study the tissue samples, i.e., the inclusion of spatial transcriptomics. Two minor changes to the patient information sheets: (1) updated information on the funding of the study and (2) an updated

				statement on this study being conducted in line with the UK General Data Protection and Regulation (UK GDPR) and data protection act. Minor change to the wording in the section of the study protocol on the questionnaires (page 20) to fully align this with the approved substantial amendment 3.
6 (MA2 – A008)	6	19th July 2022	Kurtis Garbutt	Change to where samples are stored after the completion of their analysis for the study and at the end of the study, including option to transfer to a study partner or commercial entity. Includes changes to consent form and protocol.
7 (MA3 – A009)	7	2nd December 2022	Kurtis Garbutt	Change of project endpoint to March 31 2026.
8 (MA4 – A010)	8	9th January 2024	Kurtis Garbutt	Change of project recruitment endpoint to September 30 2025, and overall project endpoint to September 30 2027.

List details of all protocol amendments here whenever a new version of the protocol is produced. This is not necessary prior to initial REC submission.