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

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3 1 Title: **FENOX: Fibroids and Endometriosis Study Oxford – A Study into the Biology of Uterine**
4
5 2 **Fibroids and Endometriosis.**

6
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21 10 **ABSTRACT**

22
23 11 **Introduction**

24
25 12 Millions of women suffer from the consequences of endometriosis and uterine fibroids, with fibroids
26
27 13 the cause for over 50% of hysterectomies in the US, and direct costs for their treatment estimated at
28
29 14 between 4 and 9 billion USD. Endometriosis commonly affects millions of women worldwide
30
31 15 predominantly during reproductive age, with severe menstrual and non-menstrual pain and
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33 16 subfertility the main symptom. Due to the 'unhappy triad' of endometriosis – lack of awareness, lack
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35 17 of clinically relevant biomarkers and the unspecific nature of symptoms – women wait on average for
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37 18 6–9 years before the definitive endometriosis diagnosis is made. Treatment options for both
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39 19 conditions are not satisfactory at the moment, especially with a view to preserving fertility for the
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41 20 women and families affected. In the FENOX study, we combine the investigation of fibroids and
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43 21 endometriosis, and plan to collect high quality tissue samples and medical data of participants over a
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45 22 time frame of 5 years after surgical intervention.

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49 24 **Methods and analysis**

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51 25 Biological samples such as blood, saliva, urine, fat, peritoneal fluid and – if found – endometrial tissue
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53 26 or fibroids as well as detailed clinical and intraoperative data will be collected from women
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55 27 participating in the study after informed consent. We plan to recruit up to 1200 participants per
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57 28 disease arm (i.e. endometriosis and uterine fibroids) over 5 years. Participants will fill in detailed and
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59 29 validated questionnaires on their medical history and quality of life, with follow-ups for 5 years. We
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30 will analyse the biological samples using state-of-the-art molecular biology methods and correlate the
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32 30 findings with the medical records and questionnaire data.

33 31 **Ethics and dissemination**

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34 Approval for this study has been granted by the South Central - Oxford B Research Ethics Committee
35 (REC No. 17/SC/0664), by the Health Research Authority (HRA), and by the Oxford University
36 Hospitals NHS Foundation Trust. The findings will be published in high-ranking journals in the field
37 and presented at national and international conferences.

38

39 **Trial registration number**

40 ISRCTN13560263; <https://doi.org/10.1186/ISRCTN13560263>

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For peer review only

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7 **ARTICLE SUMMARY**8 **Strengths and limitations of this study**

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- 10 • FENOX combines the study of endometriosis and uterine fibroids to identify the underlying
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- 11 mechanisms of both conditions.
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- 13 • The study comprises biological samples as well as comprehensive phenotypic data.
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- 15 • WERF EPHect criteria are applied to ensure the best possible standardisation.
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- 17 • The longitudinal aspect is an important feature of FENOX but this depends on uptake and
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- 18 compliance by participants.
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2223 **INTRODUCTION**24 Millions of women suffer from the consequences of endometriosis [1] and uterine fibroids [2,3].
25 These include pelvic and abdominal pain, abnormal uterine bleeding, infertility and miscarriages [4–
26 7]. As such, these conditions not only affect women and their families in their everyday lives, but also
27 have been shown to have an enormous socioeconomic impact for society in general: In the United
28 States, fibroids are cited to be the cause for over 50% of hysterectomies [8], and direct costs for their
29 treatment is estimated between 4 and 9 billion USD [9].30 Clinically relevant, non-invasive diagnostic tests including biomarkers or imaging techniques do not
31 exist for many forms of endometriosis [10–12] resulting in an average delay in diagnosis of 8-12 years.
32 Current treatment options are associated with significant side effects and risks and include hormonal
33 suppression/modification, surgical removal or, in the case of fibroids, embolization and MRI guided
34 focussed ultrasound (MRgFUS).
3536 Therefore, there exists a significant unmet clinical need to better understand the underlying
37 mechanisms of these conditions, which will enable us to develop more specific diagnostic tests and
38 will eventually lead to individualised treatment, with fewer side effects and better efficacy. To
39 achieve this goal, it is essential to collect prospective high quality, standardised clinical and intra-
40 operative data and corresponding biological samples. Our group has been at the forefront of the
41 development of standard operating procedures and questionnaires for endometriosis as part of the
42 World Endometriosis Research Foundation's (WERF) Endometriosis Phenome and Biobanking
43 Harmonisation Project (EPHect) [13–16], and we are planning to establish similar standards in uterine
44 fibroid research.
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4950 In the FENOX (Fibroids and Endometriosis in Oxford) study, we aim to improve our understanding of
51 the underlying mechanisms of endometriosis and uterine fibroids and their associated symptoms by
52 means of longitudinal observation and laboratory analyses. To achieve this, samples and clinical data
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3 78 will be collected from women undergoing surgery. These samples will be used in state-of-the-art
4 79 biomedical assays (see 'Assays') to improve our understanding of the underlying biology of these
5 80 symptoms in women with endometriosis and/or fibroids, which will lead to a better understanding of
6 81 the conditions, stratification of patient groups and tailored therapies, and the development of novel
7 82 drug targets and biomarkers for diagnosis and treatment.
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11 83 12 84 **Objectives**

13 14 15 85 *Primary objective*

- 16 86 • To identify the underlying mechanisms of endometriosis and uterine fibroids and their
17 87 associated symptoms to improve the outcome of affected women.
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20 21 22 89 *Secondary objectives*

- 23 90 • To identify novel biomarkers of endometriosis.
- 24 91 • To identify clinical subgroups of endometriosis and uterine fibroids.
- 25 92 • To understand the genetics underlying these conditions and explore the relevant
26 93 downstream molecular pathways.
- 27 94 • To investigate the relation between the presence of fibroids and the symptoms, e.g.
28 95 abnormal uterine bleeding.
- 29 96 • To identify novel drug targets.
- 30 97 • To develop models of disease progression and prediction.
- 31 98 • To investigate conditions or symptoms associated with endometriosis and/or uterine
32 99 fibroids, including: symptoms and characteristics of the female reproductive system
33 100 (characteristics of menstrual bleeding, fertility, infertility, pregnancy outcomes), pelvic as
34 101 well as non-pelvic pain conditions, metabolic phenotypes (polycystic ovarian syndrome
35 102 (PCOS), obesity and fat distribution), cardiovascular conditions and symptoms,
36 103 neuroangiogenesis and related neurological symptoms, immunological disorders, and
37 104 cancers.
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48 106 **Outcomes**

49 50 107 *Primary outcome*

51 108 We will used questionnaire data, medical records and sample analysis to investigate the genetic and
52 109 molecular basis of the pathogenesis and symptoms of endometriosis and uterine fibroids. At the end
53 110 of the recruiting period, i.e. from December 2022 onwards, the collected data and samples will be
54 111 analysed and compared between endometriosis/fibroid cases, and non-affected controls.
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3 112 *Secondary outcomes*

4 113 Prospective standardised questionnaires and samples will be collected according to EPHeC
5 114 (Endometriosis Phenome and Biobanking Harmonisation Project) standards. The correlation of data
6 115 and endometriosis status will allow us to define novel biomarkers of the disease.

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9 116 Clinical notes and questionnaires in combination with sample data will be used to define clinical
10 117 subgroups of patients.

11 118 The molecular and genetic findings will be compared against public databases of disease-relevant
12 119 molecular pathways, and *in vitro* experiments will be carried out to test hypothetical connections
13 120 between the genetics and manifestation of disease.

14 121 The blood vessels and endothelial cells will be compared between tissue from women presenting with
15 122 fibroids and those without.

16 123 The detailed comparison between tissue from women with fibroids and those without will yield
17 124 differences in terms of proteins expressed; these can then be tested as targets using known or new
18 125 drugs.

19 126 As data accumulate and genetic mechanisms become clear, hypotheses will be formed as to the likely
20 127 progression of disease. These will be tested against the reports from the follow-up questionnaires.

21 128 We will use questionnaire data, medical records and sample analysis to investigate the genetic and
22 129 molecular basis of the pathogenesis and symptoms of conditions or symptoms associated with
23 130 endometriosis and/or uterine fibroids.

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34 131 **METHODS AND ANALYSIS**

35 132 **Study design**

36 133 FENOX is a prospective study that aims to improve our understanding of the underlying mechanisms
37 134 of endometriosis and uterine fibroids and their associated symptoms by means of longitudinal
38 135 observation and laboratory analyses. Biological samples such as blood, saliva, urine, fat, peritoneal
39 136 fluid and – if found - endometrial tissue or fibroids as well as detailed clinical and intraoperative data
40 137 will be collected from women of reproductive age with and without endometriosis- and fibroid-
41 138 associated symptoms, such as pain, abnormal uterine bleeding and infertility. Women undergoing
42 139 surgery for these conditions, and women undergoing surgery for unrelated gynaecological conditions
43 140 as part of their normal clinical management will be asked to participate. All women attending clinics
44 141 receive a letter informing them of ongoing research, and eligible women will be identified initially by
45 142 research nurses or clinical staff during clinic visits. Once a woman has expressed an interest in
46 143 participating in this study, they will be consented by a member of the research team (see Fig.1).

47 144 Blood, saliva and urine will be taken prior to surgery. Tissue and peritoneal fluid (where applicable)
48 145 will be taken at the time of the scheduled surgery.

49 146 In order to determine the effect of the surgical removal of the fibroids on the local tissue, it is
50 147 necessary to take an additional endometrial biopsy after the planned surgical intervention. This
51 148 sample will be timed to synch with the same time point in the menstrual cycle that the original

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3 149 sample was taken, and thus will give us a unique insight into the biology of the conditions. During this
4 150 visit, blood and urine samples will be taken again also. Women can opt in or out of the additional
5 151 clinic visit where these samples would be taken.
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9 153 Women will be asked to complete questionnaires appropriate to their condition on paper, online or
10 154 into their electronic handheld devices (health, pain, medication and, initially, ethnicity) at different
11 155 time points. There will be a lengthy questionnaire at baseline and then shorter versions post-
12 156 operatively at 6-8 weeks, 6 months, 12 months and thereafter yearly for a total of five years after
13 157 surgical intervention.
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17 158 **Samples**

- 18 159 A. Blood samples (up to 50 ml, venepuncture), urine (micturition) and saliva (spit) will be taken
19 160 prior to surgery.
20 161 B. During surgery, tissue samples will be taken as specified below.
21 162 C. In women opting in, an additional endometrial biopsy will be taken during a follow-up visit at
22 163 least three months after surgery. This can be done in an outpatient setting, and the taking of
23 164 an endometrial biopsy in this setting using an endometrial sampling device (e.g. pipelle or
24 165 curette) is an established technique. A blood and urine sample will be taken again also.
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32 167 **Participants**

33 168 In each of the disease arms (endometriosis or fibroids), we plan to recruit up to 1200 women of
34 169 reproductive age (18 years until menopause) who are planned to undergo surgery. 800 of these will
35 170 have the condition of interest, and 400 will be having surgery for other reasons and act as controls. In
36 171 addition, we will include fibroid and uterine tissue samples collected as excess tissue (Oxford Radcliffe
37 172 Biobank, REC Ref 09/H0606/5+5), currently approximately 70 samples.
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42 174 **Inclusion criteria**

- 43 175 • The participant is willing and able to give informed consent for participation in the study.
44 176 • The participant is female and aged 18 years or above (before menopause).
45 177 • Women undergoing planned surgery (including hysterectomy) for endometriosis- and/or
46 178 fibroid associated symptoms such as abdominal pain, abnormal uterine bleeding, or for
47 179 unrelated gynaecological conditions (e.g. fertility investigation or for laparoscopic tubal
48 180 sterilisation).
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54 182 **Exclusion criteria**

55 183 The participant may not enter the study if ANY of the following apply:

- 56 184 • Women who are pregnant.
57 185 • Women who are unable to read, or to understand written or spoken English.
58 186 • History of cancer/ diagnosis of current cancer.

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3 1874 188 **Participant enrolment**6 189 *Recruitment*

8 190 After general information through a generic letter with information about ongoing research which
9 191 every patient will receive prior to her outpatient appoint, eligible women will be identified initially by
10 192 the research nurses or clinical team during clinic visits. The study research nurses will then contact
11 193 those women interested in participating in the study.

14 194 *Screening and Eligibility Assessment*

15 195 Women attending clinic appointments for endometriosis- and fibroid-associated symptoms such as
16 196 pain, abnormal uterine bleeding, and infertility will be asked to participate by clinical staff or by the
17 197 authorised study research nurses. Women undergoing surgery for these conditions, and women
18 198 undergoing surgery as part of their normal clinical management (e.g. laparoscopic tubal ligation or
19 199 hysterectomy; they would be the control patients) are eligible to participate in the study.

23 200 *Informed Consent*

24 201 Prior to giving consent, and usually during their pre-operative assessment visit, women will be given
25 202 the relevant patient information sheet and consent form to read. Written consent will be received by
26 203 a trained member of the research team.

27 204 Written versions, with verbal explanations, of the patient information sheet and the consent forms
28 205 will be presented to the participants detailing the exact nature of the study; what it will involve for
29 206 the participant; the implications and constraints of the protocol; the known side effects and any risks
30 207 involved in taking part. It will be clearly stated that the participant is free to withdraw from the study
31 208 at any time for any reason without prejudice to future care, without affecting their legal rights, and
32 209 with no obligation to give the reason for withdrawal.

33 210 The participant will be allowed as much time as wished to consider the information, and the
34 211 opportunity to question the Investigator, their GP or other independent parties to decide whether
35 212 they will participate in the study.

36 213 Written informed consent will then be obtained by means of the participant's dated signature and the
37 214 dated signature of the person who presented and obtained the informed consent. The person who
38 215 obtained the consent will be suitably qualified and experienced, and have been authorised to do so by
39 216 the Chief Investigator. A copy of the signed informed consent and the patient information sheet will
40 217 be given to the participant. The original signed form will be retained at the study site. The consent
41 218 form for this study allows for the participant declining consent for any procedure that she is not
42 219 comfortable with, while remaining eligible as a participant of the study. E.g. if a participant did not
43 220 want a uterine biopsy used in the study, she would not initial the corresponding box on the consent
44 221 form and insert 'No' instead.

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3 223 **Study settings**
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5 224 *Baseline Assessments*
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7 225 Consented participants will be asked by the research team to complete a baseline questionnaire
8 226 before their surgery as appropriate to their condition. This may be sent to them e.g. via mail or e-mail
9 227 before surgery or given to them in paper form in the clinic. Alternatively, participants have the option
10 228 to complete an online version of the questionnaire or they may fill in the questionnaire on their hand-
11 229 held device. Each participant will receive a unique login for the online questionnaires.

12 230 The questionnaire data will be withheld from the research team until written informed consent is
13 231 obtained, and destroyed if this is not granted.

14 232 All participants will be sent further questionnaires at different time points (approximately 6-8 weeks,
15 233 6 months, 12 months and then yearly for 5 years after surgery). Participants may be reminded (twice,
16 234 maximally) to return completed questionnaires via mail, email, phone or text or similar.

17 235 On the day of surgery, they will be asked to provide a mid-stream urine and a saliva sample. In
18 236 addition, blood will be collected by peripheral venepuncture. The procedures will be explained to the
19 237 women again and they will be given the opportunity to ask questions. Assessment of the presence
20 238 and extent of disease will be performed by the operating surgeon.

21 239 *Samples that may be taken at time of surgical procedures are as follows:*
22 240

23 241 1. *Laparoscopy for suspected endometriosis:*

24 242 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue if present,
25 243 peritoneal biopsy.
26 244

27 245 2. *Laparoscopy for uterine fibroids:*

28 246 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue (if present),
29 247 peritoneal biopsy, fibroid tissue, myometrial biopsy.
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31 249 3. *Laparotomy for uterine fibroids:*

32 250 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue if present,
33 251 peritoneal biopsy, fibroid tissue, myometrial biopsy.
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35 253 4. *Trans-cervical resection of uterine fibroids (TCRF):*

36 254 Endometrial biopsy, fibroid tissue, myometrial biopsy
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3 256 5. *Laparoscopy for tubal sterilisation (Controls):*

4 257 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, peritoneal biopsy, myometrial
5 258 biopsy

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10 260 6. *Hysterectomy:*

11 261 Whole uterus as excess tissue from women with fibroids as well as from controls without fibroids,
12 262 who undergo surgery for other indications (such as heavy menstrual bleeding, or pain), peritoneal
13 263 fluid aspiration, fat tissue biopsy and peritoneal biopsy.

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17 265 Women undergoing hysterectomy for benign causes such as abnormal uterine bleeding or pain will be
18 266 asked to donate part of their uterus for research. Hysterectomy specimens are excess tissue and
19 267 would be discarded otherwise.

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23 269 During surgery, the surgeon will record digital photographs of the inside of the abdomen and/or
24 270 uterine cavity as part of routine clinical care, which will also be stored on a secure server identified by
25 271 the participant's study ID. Intraoperative findings will be recorded by the surgeon and anonymised
26 272 data collected.

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31 274 *Subsequent Visits*

32 275 Unless participants underwent a hysterectomy, all included women will be asked to contact the study
33 276 team when the next menstrual period after the procedure started, in order to calculate the length of
34 277 that cycle. This is important in order to account for the changes that occur in the uterus during the
35 278 menstrual cycle, and to enable us to distinguish between the effects of the cycle and the disease.

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38 280 One subsequent visit will be made by participants who consent to this. They will have another
39 281 endometrial sample taken at least three months after the surgical intervention in an outpatient
40 282 setting. The taking of an endometrial biopsy in this setting using an endometrial sampling device (e.g.
41 283 pipelle or curette) is an established technique, takes approximately 30 minutes, and there is only a
42 284 minimal risk of bleeding. In addition, blood and urine samples will be taken also. Pregnant women will
43 285 not be eligible for the subsequent visits.

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45 286 All women will be contacted by a member of the medical or study team and asked to fill in further
46 287 questionnaires at different follow-up time points (approximately 6-8 weeks, 6 months, 12 months and
47 288 yearly thereafter for a total of 5 years). Reminders will be sent twice, maximally. Women will also be
48 289 asked if they can be contacted in the future for any further studies approved by an ethics committee.

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51 291 *Sample Handling*

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

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

302 We also intend to use the endometrium and endometriotic samples, one of the blood samples and fat
303 samples to look for genetic factors and molecular pathways that can lead to endometriosis or uterine
304 fibroids. The samples for this analysis will also be anonymised so that we do not know specifically
305 which patient they came from. However, all samples are identifiable with printed label and location
306 detail, participant ID, sample type and colour-coded cap.

307 *Blood:*

308 50 mL. These are divided into (at least) EDTA- (2 x 9 mL) and heparin-treated samples (6 mL, both
309 anti-coagulation), serum (SST, 5 mL) and a plain blood sample of 5 mL. The different vials are colour-
310 coded and frozen at -80°C.

311 *Urine:*

312 20 mL. Half of the sample will be used to test for glucose by specific gravity assay, the other half will
313 be stored for the study. One aliquot of 5 mL is frozen directly at -80°C; 1 mL is centrifuged at 300 g,
314 and 5 aliquots a 200 µL of cell-free supernatant are frozen at -80°C.

315 *Saliva:*

316 A spit sample of approximately 1 mL is taken on ice. One aliquot of 200 µL is frozen at -80°C, the rest
317 is centrifuged at 300 g and 2 x 200 µL of cell-free supernatant are frozen at -80°C. One aliquot of 50 µL
318 of cell-free supernatant is combined with 200 µL of RNA-preserving buffer (RNA later, Qiagen,
319 Germany) and then frozen at -80°C.

320 *Peritoneal fluid:*

321 During surgery, the peritoneal fluid will be collected on ice. Depending on the volume (up to 15 mL),
322 an aliquot will be centrifuged at 300 g, and the pellet (cells) stored at -80°C for further analysis. The
323 cell-free supernatant will also be stored at -80°C.

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3 324 *Endometrium (e.g. pipelle or curette), endometrial lesions (peritoneum), abdominal fat, myometrium,*
4 *fibroid tissue:*

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6 326 All tissue will be collected on ice and divided for storage at -80°C and – after fixing in
7 paraformaldehyde and ethanol – at room temperature. Parts of fresh tissues will be used for culturing
8 experiments, in order to test compounds, drugs or similar agents on primary cells.
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11 329 *Hysterectomy:*

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13 330 In agreement with the local pathologist, whole uteri will be taken on ice and used for perfusion
14 experiments within 24 hours before being transferred to pathology. Tissue samples of myometrium,
15 331 endometrium, fibroid and fibroid-associated vasculature (if present) will be taken and stored at -80°C
16 332 and – after fixing in paraformaldehyde and ethanol – at room temperature as the other tissue
17 333 samples above.
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21 335 *Assays*

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24 336 *RNA analysis*

25 337 RNA from each sample will be isolated by standard methods. Gene expression studies will be carried
26 338 out between cases and controls (e.g. endometriosis vs non-endometriosis patients, or fibroid bearing
27 339 women vs women without fibroids) using quantitative real-time PCR assays, whole RNA sequencing
28 340 methods and RNA microarrays.
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32 341 *Protein analysis*

33 342 Proteins will be extracted from tissue samples using standard methods. The expression and amount
34 343 of proteins will be analysed by immunoblotting for specific proteins of interest, and by proteomics
35 344 methods using the MALDI/SELDI platform. Tissue sections will be used in standard
36 345 immunohistochemistry to detect the expression of markers of interest *in situ*.

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39 346 *Cells*

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41 347 Fresh tissue will be dissociated into single cell suspensions. From these, the diverse cell types (e.g.
42 348 endothelial cells) will be grown in incubators *in vitro* in order to study differences in cell behaviour
43 349 between cases and controls, and to test compounds and drugs. Cells will be analysed by microscopy,
44 350 flow cytometry and immunocytochemistry methods.

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47 351 Similarly, cells isolated from peritoneal fluid or blood will be analysed using these methods.

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50 352 *Secretome analysis (perfusion)*

51 353 Whole uteri with and without fibroids will be perfused with a suitable buffer for up to 8 hours. The
52 354 perfusate will be analysed by proteomics methods (see above) to detect factors secreted by the
53 355 fibroids.
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356 *Microscopy*

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

362

363 **Discontinuation/Withdrawal of Participants from Study**

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

- 367 • Pregnancy
- 368 • Ineligibility (either arising during the study or retrospectively, having been missed at
369 screening)
- 370 • Withdrawal of Consent
- 371 • Loss to follow up
- 372 • Loss of mental capacity

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

375 Withdrawn participants will not be replaced.

376 The reason for withdrawal will be recorded in the CRF.

377 *Definition of End of Study*

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

380 **Patient and Public Involvement**

381 FENOX was built on experience and feedback we received from patients and research nurses during a
382 previous study (ENDOX[17]). In addition, the research objectives were set in accordance with research
383 priorities identified through the James Lind Alliance Priority Setting Partnership (PSP) for
384 endometriosis, in which we participate[18]. The James Lind Alliance brings patients, carers and
385 clinicians together in Priority Setting Partnerships (PSPs) to identify and prioritise the Top 10
386 unanswered questions or evidence uncertainties that they agree are the most important.

387 **INTERVENTIONS**

388 **Non-clinical Interventions**

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3 389 *Questionnaires:*

4 390 As appropriate for their study arm – endometriosis or uterine fibroids – participants will complete
5 391 specific questionnaires about general health, pain sensitivity, medication and menstrual history
6 392 before their surgery. Additionally, those women with endometriosis will be asked to complete the
7 393 Endometriosis Health Profile (EHP-30) Questionnaire, while the questionnaire for women with
8 394 fibroids contains a section on their quality of life (UFS-QoL[19]). Currently, the clinical questionnaires
9 395 are completed by participants on paper; in the future, the aim is that the questionnaires can be
10 396 completed via a handheld device or via the internet directly onto a secure server. For this, women will
11 397 receive a pre-trial study number and login information. The follow-up questionnaires ask about
12 398 symptoms and changes in menstrual history as relevant. The control groups would be given the same
13 399 questionnaires as the women with the respective condition, and asked to omit questions that are not
14 400 applicable to them.
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24 402 *Medical records:*

25 403 We will obtain clinical data (menstrual cycle phase, medication, pain and menstrual bleeding status,
26 404 photos from surgery) from the patients' medical records.

27 405 **Clinical Interventions**

28 406 *Venepuncture:*

29 407 Taken by an appropriately trained member of the clinical or research staff.

30 408 *Collection of other bodily fluid sample:*

31 409 Urine and saliva samples donated by the patient and sample prepared and analysed by a member of
32 410 the investigative team.

33 411 *Tissue collection:*

34 412 Tissue/fluid (e.g. fibroids if present) will be collected as part of routine surgical management apart
35 413 from:

36 414 *Laparoscopy:*

37 415 Peritoneal fluid will be aspirated, biopsies from endometrium (e.g. pipelle or curette), abdominal fat
38 416 tissue, myometrium, and peritoneum (excision) will be taken during surgery.

39 417 *Additional Risk:*

40 418 Minor bleeding, uterine perforation (<1%), additional length of surgery by 5 min.

41 419 *Myomectomy/hysterectomy:*

42 420 Endometrial, myometrial and/or fibroid tissue biopsies will be taken during surgery. Hysterectomy
43 421 samples will be used in structural analysis assays *ex vivo* in close discussion with the clinical
44 422 pathologists.

45 423 *Additional Risk:* Minor bleeding, uterine perforation (<1%), additional length of surgery by 5 min.

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3 424 *Trans-cervical resection of fibroids:*

4 425 Endometrial and myometrial biopsies will be taken during surgery.

5 426 Additional Risk: Minor bleeding, uterine perforation (<1%), additional length of surgery by 5 min.

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8 427 *Additionally:*

9 428 In women opting in, an additional endometrial biopsy will be taken during a follow-up visit in an
10 429 outpatient setting. The biopsy of the endometrium is a simple, routine procedure and takes about 30
11 430 minutes.

12 431 Additional risk: Minor bleeding, uterine perforation (<1%), short period of discomfort.

13 432

14 433 Women will be asked to consent to the use of samples and clinical data collected as part of this
15 434 research and in future research. Women, if they consent, will potentially be contacted for future
16 435 studies approved by an ethics committee.

17 436

18 437 **Adverse events**

19 438 For this study, it is conceivable that additional procedures may result in bleeding. However, if this
20 439 resulted in a scenario mentioned below, it would constitute an SAE and needed to be reported to the
21 440 sponsor.

22 441

23 442 A serious adverse event is any untoward medical occurrence that:

- 24 443
- 25 444 • results in death
 - 26 445 • is life-threatening
 - 27 446 • requires inpatient hospitalisation or prolongation of existing hospitalisation
 - 28 447 • results in persistent or significant disability/incapacity
 - 29 448 • consists of a congenital anomaly or birth defect

30 449 Other 'important medical events' may also be considered serious if they jeopardise the participant or
31 450 require an intervention to prevent one of the above consequences.

32 451 NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the
33 452 participant was at risk of death at the time of the event; it does not refer to an event which
34 453 hypothetically might have caused death if it were more severe.

35 454

36 455 A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a
37 456 favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related'
38 457 (resulted from administration of any of the research procedures) and 'unexpected' in relation to
39 458 those procedures. Reports of related and unexpected SAEs should be submitted within 15 working
40 459 days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse
41 460 event form (see HRA website).

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461 **Description of Statistical Methods**

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

468 **The Number of Participants**

469 It is now recognised that both endometriosis and uterine fibroids are very heterogeneous conditions.
470 Our previous studies[20,21] and systematic reviews[22,23] have clearly identified a lack of sufficiently
471 powered studies. Multiple large-scale research collaborations are currently in place investigating
472 different aspects of endometriosis[24], and we plan similar efforts for uterine fibroids. Therefore,
473 large patient numbers are needed.

474 The Endometriosis CaRe Centre at Oxford is the UK's largest endometriosis centre. Similarly, as a
475 tertiary referral centre, we see many women with fibroid-associated symptoms. As a result, we have
476 the unique opportunity to collect large amounts of data and samples, which is essential to produce
477 clinically meaningful outputs. Given our current recruitment rate (endometriosis: 100/year, uterine
478 fibroids, 200/year) we estimate the recruitment of approximately 2×1200 women over the course of
479 the study (800 endometriosis patients + 400 non-endometriotic controls, 800 fibroid patients + 400
480 non-fibroid controls). Fibroids already collected as excess tissue under the Oxford Radcliffe Biobank
481 (ORB, REC Ref 09/H0606/5+5) will also be included in this study, currently approximately 70 samples.

482 **Analysis of Outcome Measures**

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

485 Laboratory data will be analysed using assay-specific software packages employing univariate and
486 multivariate pattern recognition methods (e.g. principal component analysis, partial least squares,
487 stochastic neighbour embedding algorithms) between sample groups. Correlation with questionnaire
488 data will allow us to validate prospective markers of disease. In addition, we will use laboratory data
489 to predict disease severity (revised American Fertility Score), quality of life (EHP-30), pain measures
490 and improvement of symptoms as per follow-up questionnaires. For the multivariate predictive
491 methods, a test set of approximately 30% of each treatment group will be selected at random. This
492 may be selected in a stratified method and exclude patients that have particularly extreme values.
493 Patients not included in the test set will make up the training set. Models will then be built on the
494 training set and assessed for predictability on the test set.

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

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3 499 **DATA MANAGEMENT**

4 500 **Access to Data**

5 501 Direct access will be granted to authorised representatives from the Sponsor and host institution for
6 502 monitoring and/or audit of the study to ensure compliance with regulations.

7 503 **Data Recording and Record Keeping**

8 504 Each participant will receive a unique study number, which will then be used throughout the study. A
9 505 study master sheet linking patient identifiable data (name, DOB, hospital and NHS numbers) with the
10 506 unique study number will be kept and password protected on the University of Oxford's High
11 507 Compliance server with authorised access and in a file separate from the main study file. Hard copy
12 508 study documents will be kept in a locked room at each participating centre. Research data will
13 509 therefore be using non-identifiable data, and all records will be identified only by this study number.
14 510 All study data will be entered on a desktop computer into a program such as Microsoft EXCEL or
15 511 Sapphire (Labvantage) using password protection. The participants will be identified by study number
16 512 in any database. The name and any other identifying details will NOT be included in any electronic file
17 513 of study data.

18 514 Where participants consent, coded genetic data and limited relevant details including, age, gender,
19 515 information about body habitus, biochemistry etc. can also be made available to collaborators and to
20 516 the National Institute for Health Research (NIHR) Bioresource (<http://bioresource.nihr.ac.uk/>), a panel
21 517 of thousands of volunteers, who are willing to be approached to participate in research studies
22 518 investigating the links between genes, the environment, health and disease.

23 519 **QUALITY ASSURANCE PROCEDURES**

24 520 The study may be monitored, or audited in accordance with the current approved protocol, GCP,
25 521 relevant regulations and standard operating procedures.

26 522 **ETHICAL AND REGULATORY CONSIDERATIONS**

27 523 **Declaration of Helsinki**

28 524 This study will be conducted in accordance with the principles of the Declaration of Helsinki.

29 525 **Guidelines for Good Clinical Practice**

30 526 The Investigator will ensure that this study is conducted in accordance with relevant regulations and
31 527 with Good Clinical Practice.

32 528 **Approvals**

33 529 The protocol, informed consent form, participant information sheet and any proposed advertising
34 530 material will be submitted to an appropriate Research Ethics Committee (REC), and HRA for written
35 531 approval.

36 532 The Investigator will submit and, where necessary, obtain approval from the above parties for all
37 533 substantial amendments to the original approved documents.

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3 534 **Reporting**

4 535 The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the
5 536 REC Committee, HRA (where required) host organisation and Sponsor. In addition, an End of Study
6 537 notification and final report will be submitted to the same parties.

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9 538 **Participant Confidentiality**

10 539 The study staff will ensure that the participants' anonymity is maintained. The participants will be
11 540 identified only by a participant ID number on all study documents and any electronic database, with
12 541 the exception of the CRF, where participant initials may be added. All documents will be stored
13 542 securely and only accessible by study staff and authorised personnel. The study will comply with the
14 543 Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

15 544 **Expenses and Benefits**

16 545 There will be no payments made to study participants.

17 546 **Other Ethical Considerations**

18 547 Participants unable to consent for themselves will not be included in the study.

19 548 Patients under clinical management for infertility will be approached in a most sensitive manner by
20 549 our experienced and well-trained team. It is unlikely that our genetic analysis of the participants will
21 550 reveal anything relevant beyond their normal clinical care so we do not plan to report any such
22 551 findings to them or their GPs.

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34 553 **DISCUSSION**

35 554 This study has been designed to address both uterine fibroids and endometriosis as conditions that
36 555 both affect the female reproductive system and pose similar problems with regards to pain
37 556 treatment, fertility and quality of life. By combining the patient collectives into one study, we hope to
38 557 make use of synergies between the investigations of the two conditions, in addition to the apparent
39 558 comorbidity between endometriosis patients and those with uterine fibroids [25,26]. Uniquely, the
40 559 study protocol allows for the sampling of endometrium on a follow-up visit, which will allow for the
41 560 assessment of the local, molecular effects of treatment within the same participant.

42 561 FENOX has been designed with the EPHect principles [13–16] in mind to ensure standardisation and
43 562 reproducibility, and thus should deliver high-quality datasets that will be useful and comparable
44 563 between centres. We are currently expanding the collection of samples to sites outside of Oxford,
45 564 with a view to make FENOX a multi-centre study within the UK eventually.

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9 574 Oxford, in preparing the study protocol.

11
12 575 *Contributors*

14 576 TTT wrote the study protocol with KTZ and CMB. CH, KB and ES consented participants and collected
15 577 samples. HMN, KSS and TTT processed samples. KG, EJJ and CC documented samples and designed
17 578 electronic questionnaires. SM determined menstrual cycle stages by histology. KTZ and CMB
18 579 conceived the study.

20
21 580 *Funding*

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26 585 Women's & Reproductive Health under the Oxford/Bayer-Alliance for Women's Health.

29
30
31 586 *Competing interests*

32 587 CMB and KTZ received research grants from Bayer Healthcare, Volition RX, MDNA Life Sciences and
33 588 Roche Diagnostics. The study is funded by the Nuffield Department of Women's & Reproductive
34 589 Health.

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38 590 *Patient consent*

39 591 See above, *informed consent*

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42 592 *Ethics approval*

43 593 Approval has been granted by the South Central - Oxford B Research Ethics Committee (REC No.
44 594 17/SC/0664), by the Health Research Authority (HRA), and by the Oxford University Hospitals NHS
45 595 Foundation Trust.

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49 596 *Provenance and peer review*

50 597 The FENOX protocol was based upon the previous ENDOX study (REC reference 09/H0604/58). It was
51 598 originally approved in February 2018, with amendments approved in April 2019.

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53
54 599 *Open access*

55 600 The study protocol will be published under a Creative Commons licence.
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For peer review only

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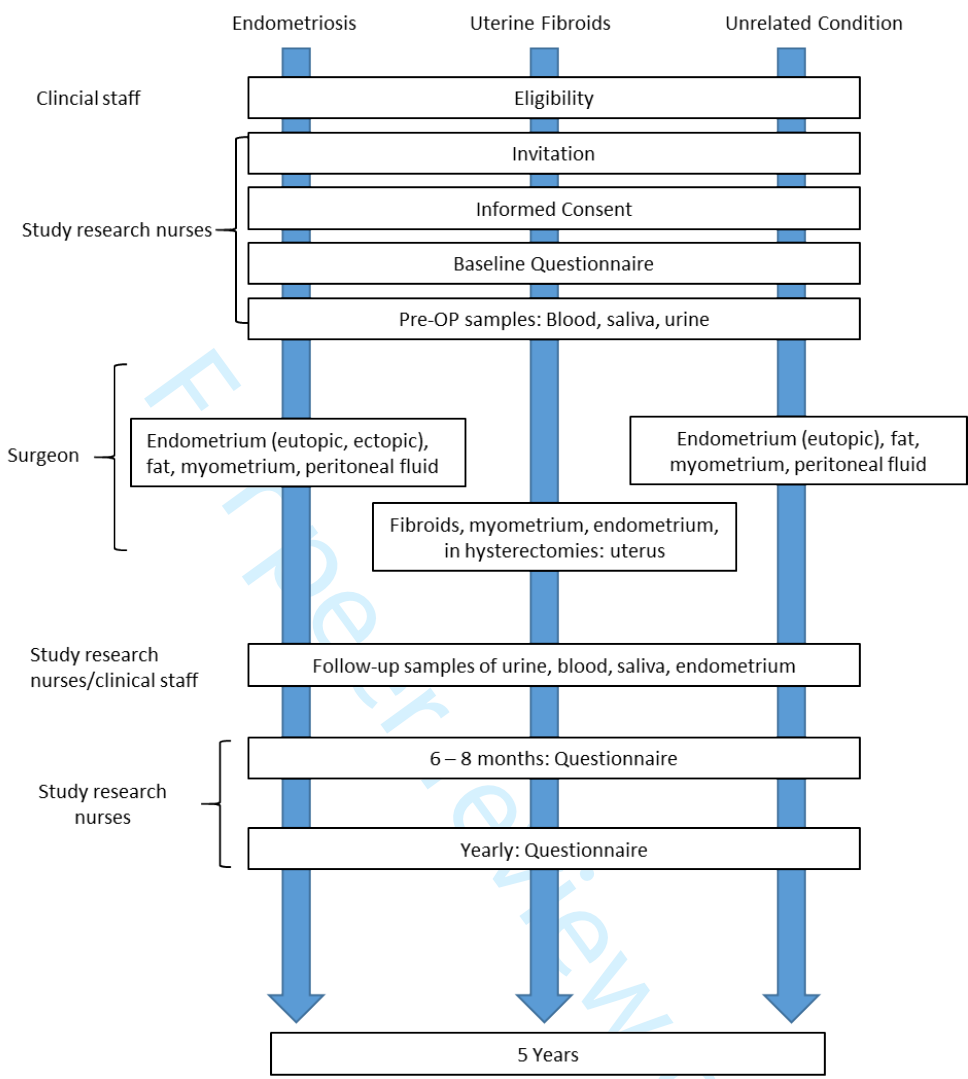


Figure 1: Flowchart of data and tissue sampling.

BMJ Open

A protocol for a longitudinal, prospective cohort study investigating the biology of uterine fibroids and endometriosis, and patients' quality of life – the FENOX study.

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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Genetics and genomics, Reproductive medicine, Epidemiology
Keywords:	GYNAECOLOGY, Reproductive medicine < GYNAECOLOGY, Subfertility < GYNAECOLOGY, REPRODUCTIVE MEDICINE, SURGERY

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5 2 Title: **A protocol for a longitudinal, prospective cohort study investigating the biology of uterine**
6
7 3 **fibroids and endometriosis, and patients' quality of life – the FENOX study.**

8
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24
25 12 **ABSTRACT**

26
27 13 **Introduction**

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29 14 Millions of women suffer from the consequences of endometriosis and uterine fibroids, with fibroids
30
31 15 the cause for over 50% of hysterectomies in the US, and direct costs for their treatment estimated at
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33 16 between 4 and 9 billion USD. Endometriosis commonly affects millions of women worldwide
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35 17 predominantly during reproductive age, with severe menstrual and non-menstrual pain and subfertility
36
37 18 the main symptoms. Due to the 'unhappy triad' of endometriosis – lack of awareness, lack of clinically
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39 19 relevant biomarkers and the unspecific nature of symptoms – women wait on average for 8–12 years
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41 20 before the definitive endometriosis diagnosis is made. Treatment options for both conditions are not
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43 21 satisfactory at the moment, especially with a view to preserving fertility for the women and families
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45 22 affected. In the FENOX study, we combine the investigation of fibroids and endometriosis, and plan to
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47 23 collect high quality tissue samples and medical data of participants over a time frame of 5 years after
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49 24 surgical intervention.

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51 25
52 26 **Methods and analysis**

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54 27 Biological samples such as blood, saliva, urine, fat, peritoneal fluid and – if found – endometrial tissue
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56 28 or fibroids as well as detailed clinical and intraoperative data will be collected from women undergoing
57
58 29 surgery and participating in the study after informed consent. We plan to recruit up to 1200 participants
59
60 30 per disease arm (i.e. endometriosis and uterine fibroids) over 5 years. Participants will fill in detailed
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32 31 and validated questionnaires on their medical history and quality of life, with follow-ups for 5 years.
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34 32 Enrolment started on 2nd April 2018, and FENOX will close on 31st March 2028. We will analyse the
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36 33 biological samples using state-of-the-art molecular biology methods and correlate the findings with the
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38 34 medical records and questionnaire data.

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5 36 **Ethics and dissemination**6
7 37 Approval for this study has been granted by the South Central - Oxford B Research Ethics Committee
8 38 (REC No. 17/SC/0664), by the Health Research Authority (HRA), and by the Oxford University Hospitals
9
10 39 NHS Foundation Trust. The findings will be published in high-ranking journals in the field and presented
11 40 at national and international conferences.
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14 42 **Trial registration number**15
16 43 ISRCTN13560263; <https://doi.org/10.1186/ISRCTN13560263>
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For peer review only

ARTICLE SUMMARY

Strengths and limitations of this study

- FENOX combines the study of endometriosis and uterine fibroids to identify the underlying mechanisms of both conditions.
- The study comprises biological samples as well as comprehensive phenotypic data.
- WERF EPHect criteria are applied to ensure the best possible standardisation.
- The longitudinal aspect is an important feature of FENOX but this depends on uptake and compliance by participants.
- The control group comprises of women undergoing surgery for gynaecological indications other than endometriosis or uterine fibroids; thus, they are not completely healthy controls. This is a limitation of this study. However, we cannot ethically source tissue samples from healthy individuals.

INTRODUCTION

Millions of women suffer from the consequences of endometriosis [1,2] and uterine fibroids [3,4]. These include pelvic and abdominal pain, abnormal uterine bleeding, infertility and miscarriages [5–8]. 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 [9], and direct costs for their treatment is estimated between 4 and 9 billion USD [10].

Clinically relevant, non-invasive diagnostic tests including biomarkers or imaging techniques do not exist for many forms of endometriosis [11–13] 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, 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) [14–17], 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 '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.

Objectives

Primary objective

- To identify the underlying mechanisms of endometriosis and uterine fibroids and their associated symptoms to improve the outcome of affected women.

Secondary objectives

- To identify novel biomarkers of endometriosis.
- To identify clinical subgroups of endometriosis and uterine fibroids.
- To understand the genetics underlying these conditions and explore the relevant downstream molecular pathways.
- To investigate the relation between the presence of fibroids and the symptoms, e.g. abnormal uterine bleeding.
- To identify novel drug targets.
- To develop models of disease progression and prediction.
- 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.

Outcomes

Primary outcome

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 2022 onwards, the collected data and samples will be analysed and compared between endometriosis/fibroid cases, and non-affected controls.

Secondary outcomes

Prospective standardised questionnaires and samples will be collected according to EPHeC (Endometriosis Phenome and Biobanking Harmonisation Project) standards. The correlation of cellular,

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118 molecular and genetic data and endometriosis status will allow us to define novel biomarkers of the
119 disease.

120 Clinical notes and questionnaires in combination with sample data will be used to define clinical
121 subgroups of patients.

122 The molecular and genetic findings will be compared against public databases of disease-relevant
123 molecular pathways, and *in vitro* experiments will be carried out to test hypothetical connections
124 between the genetics and manifestation of disease.

125 The blood vessels and endothelial cells will be compared between tissue from women presenting with
126 fibroids and those without.

127 The detailed comparison between tissue from women with fibroids and those without will yield
128 differences in terms of proteins expressed; these can then be tested as targets using known or new
129 drugs.

130 As data accumulate and genetic mechanisms become clear, hypotheses will be formed as to the likely
131 progression of disease. These will be tested against the reports from the follow-up questionnaires.

132 We will use questionnaire data, medical records and sample analysis to investigate the genetic and
133 molecular basis of the pathogenesis and symptoms of conditions or symptoms associated with
134 endometriosis and/or uterine fibroids.

135 **METHODS AND ANALYSIS**

136 **Study design**

137 FENOX is a prospective study that aims to improve our understanding of the underlying mechanisms of
138 endometriosis and uterine fibroids and their associated symptoms by means of longitudinal
139 observation and laboratory analyses. Biological samples such as blood, saliva, urine, fat, peritoneal
140 fluid and – if found - endometriosis tissue or fibroids as well as detailed clinical and intraoperative data
141 will be collected from women of reproductive age with and without endometriosis- and fibroid-
142 associated symptoms, such as pain, abnormal uterine bleeding and infertility. Women undergoing
143 surgery for these conditions, and women undergoing surgery for unrelated gynaecological conditions
144 as part of their normal clinical management will be asked to participate (Fig. 1). An incidental diagnosis
145 of endometriosis or uterine fibroids will lead to the patients' inclusion into the relevant case groups. All
146 women attending clinics receive a letter informing them of ongoing research, and eligible women will
147 be identified initially by research nurses or clinical staff during clinic visits. Once a woman has expressed
148 an interest in participating in this study, they will be consented by a member of the research team
149 (flowchart, Fig. 2).

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

152 In order to determine the effect of the surgical removal of the fibroids on the local tissue, it is necessary
153 to take an additional endometrial biopsy after the planned surgical intervention. This sample will be
154 timed to synch with the same time point in the menstrual cycle that the original sample was taken, and

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3 155 thus will give us a unique insight into the biology of the conditions. During this visit, blood and urine
4 156 samples will be taken again also. Women can opt in or out of the additional clinic visit where these
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6 157 samples would be taken.
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9 159 Women will be asked to complete questionnaires on paper, online or into their electronic handheld
10 160 devices (health, pain, medication and, initially, ethnicity) at different time points. There will be a lengthy
11 161 questionnaire at baseline before surgery (taking an estimated 45 minutes to complete), and shorter
12 162 versions (taking up to 30 minutes to complete) post-operatively at 6-8 weeks, 6 months, 12 months and
13
14 163 thereafter yearly for a total of five years after surgical intervention.
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17 164 **Samples**

- 18 165 A. Blood samples (up to 50 ml, venepuncture), urine (micturition) and saliva (spit) will be taken
19 166 prior to surgery.
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21 167 B. During surgery, tissue samples will be taken as specified below.
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23 168 C. In women opting in, an additional endometrial biopsy will be taken during a follow-up visit at
24 169 least three months after surgery. This can be done in an outpatient setting, and the taking of
25 170 an endometrial biopsy in this setting using an endometrial sampling device (e.g. pipelle or
26 171 curette) is an established technique. A blood and urine sample will be taken again also.
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30 173 **Participants**

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32 174 In each of the disease arms (endometriosis or fibroids), we plan to recruit up to 1200 women of
33 175 reproductive age (18 years until menopause) who are planned to undergo surgery. 800 of these will
34 176 have the condition of interest, and 400 will be having surgery for other reasons and act as controls. In
35 177 addition, we will include fibroid and uterine tissue samples collected as excess tissue (Oxford Radcliffe
36 178 Biobank, REC Ref 09/H0606/5+5), currently approximately 70 samples.
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40 180 **Inclusion criteria**

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43 181 • The participant is willing and able to give informed consent for participation in the study.
44 182 • The participant is female and aged 18 years or above (before menopause).
45 183 • Women undergoing planned surgery (including hysterectomy) for endometriosis- and/or
46 184 fibroid associated symptoms such as abdominal pain, abnormal uterine bleeding, or for
47 185 unrelated gynaecological conditions (e.g. fertility investigation or for laparoscopic tubal
48 186 sterilisation).
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52 188 **Exclusion criteria**

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54 189 The participant may not enter the study if ANY of the following apply:

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56 190 • Women who are pregnant.
57 191 • Women who are unable to read, or to understand written or spoken English.
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59 192 • History of cancer/ diagnosis of current cancer.
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228**Participant enrolment***Recruitment*

After general information through a generic letter with information about ongoing research, which every patient will receive prior to her outpatient appointment, 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.

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.

Informed Consent

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.

Written informed consent will then be obtained by means of the participant's dated signature and the dated signature of the person who presented and obtained the informed consent. The person who obtained the consent will be suitably qualified and experienced, and will have been authorised to do so by the Chief Investigator. A copy of the signed informed consent and the patient information sheet will be given to the participant. The original signed form will be retained at the study site. 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. E.g. 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.

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3 229 **Study settings**
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6 230 *Baseline Assessments*

7 231 Consented participants will be asked by the research team to complete a baseline questionnaire before
8 232 their surgery. This may be sent to them e.g. via mail or e-mail before surgery or given to them in paper
9 233 form in the clinic. Alternatively, participants have the option to complete an online version of the
10 234 questionnaire or they may fill in the questionnaire on their hand-held device. Each participant will
11 235 receive a unique login for the online questionnaires.

12 236 The questionnaire data will be withheld from the research team until written informed consent is
13 237 obtained and will be destroyed if this is not granted.

14 238 All participants will be sent further questionnaires at different time points (approximately 6-8 weeks, 6
15 239 months, 12 months and then yearly for 5 years after surgery). Participants may be reminded (twice,
16 240 maximally) to return completed questionnaires via mail, email, phone or text or similar.

17 241 On the day of surgery, they will be asked to provide a mid-stream urine and a saliva sample. In addition,
18 242 blood will be collected by peripheral venepuncture. The procedures will be explained to the women
19 243 again and they will be given the opportunity to ask questions. Assessment of the presence and extent
20 244 of disease will be performed by the operating surgeon.

21 245 *Samples that may be taken at time of surgical procedures are as follows:*
22 246

23 247 1. *Laparoscopy for suspected endometriosis:*

24 248 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue if present,
25 249 peritoneal biopsy.
26 250

27 251 2. *Laparoscopy for uterine fibroids:*

28 252 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue (if present),
29 253 peritoneal biopsy, fibroid tissue, myometrial biopsy.
30 254

31 255 3. *Laparotomy for uterine fibroids:*

32 256 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue if present,
33 257 peritoneal biopsy, fibroid tissue, myometrial biopsy.
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35 259 4. *Trans-cervical resection of uterine fibroids (TCRF):*

36 260 Endometrial biopsy, fibroid tissue, myometrial biopsy
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262 5. *Laparoscopy for tubal sterilisation (Controls):*

263 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, peritoneal biopsy, myometrial biopsy
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265 6. *Hysterectomy:*

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

269
270 Women undergoing hysterectomy for benign causes such as abnormal uterine bleeding or pain will be
271 asked to donate part of their uterus for research. Hysterectomy specimens are excess tissue and would
272 be discarded otherwise.

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

278

279 *Subsequent Visits*

280 Unless participants underwent a hysterectomy, all included women will be asked to contact the study
281 team when the next menstrual period after the procedure started. Together with the last menstrual
282 period (LMP) date given at the time of the procedure, this date will be used to calculate the length of
283 the cycle. This is important in order to account for the changes that occur in the uterus during the
284 menstrual cycle, and to enable us to distinguish between the effects of the cycle and the disease.

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

292 All women will be contacted by a member of the medical or study team and asked to fill in further
293 questionnaires at different follow-up time points (approximately 6-8 weeks, 6 months, 12 months and
294 yearly thereafter for a total of 5 years). Reminders will be sent twice, maximally. Women will also be
295 asked if they can be contacted in the future for any further studies approved by an ethics committee.

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297 *Sample Handling*

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3 298 Samples will be obtained according to WERF/EPHect guidelines[14–17] by the interventions listed
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5 299 below. Only the study team will have access. Biological samples will be stored at -20°C or at -80°C for
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7 300 use in current and future studies until exhausted. Disease and control samples will be stored under the
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9 301 same conditions. For the purposes of this study, samples may be analysed at Oxford, or they may be
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11 302 transferred to a third party/study collaborator, including industrial partners, for analysis at their facility.
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13 303 If participants agree, samples will be moved to a Research Tissue Bank at the end of the study or stored
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15 304 and used in future ethically approved studies. They would be made available in anonymised form.
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17 305 To investigate the relationship between uterine fibroids and symptoms such as abnormal uterine
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19 306 bleeding and pain, we aim to collect endometrial and myometrial samples alongside the fibroids
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21 307 themselves, to be able to detect the effects of fibroids on their surroundings.
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23 308 We also intend to use the endometrium and endometriotic samples, one of the blood samples and fat
24
25 309 samples to look for genetic factors and molecular pathways that can lead to endometriosis or uterine
26
27 310 fibroids. The samples for this analysis will also be anonymised so that we do not know specifically which
28
29 311 patient they came from. However, all samples are identifiable with printed label and location detail,
30
31 312 participant ID, sample type and colour-coded cap.

313 *Blood:*

314 50 mL. These are divided into (at least) EDTA- (2 x 9 mL) and heparin-treated samples (2 x 6 mL, both
315 anti-coagulation), serum (2 x SST, 5 mL) and two plain blood samples of 5 mL. The different vials are
316 colour-coded and frozen at -80°C.

317 *Urine:*

318 20 mL. Half of the sample will be used to test for glucose by specific gravity assay, the other half will be
319 stored for the study. One aliquot of 5 mL is frozen directly at -80°C; 1 mL is centrifuged at 300 g, and 5
320 aliquots a 200 µL of cell-free supernatant are frozen at -80°C.

321 *Saliva:*

322 A spit sample of approximately 1 mL is taken on ice. One aliquot of 200 µL is frozen at -80°C, the rest is
323 centrifuged at 300 g and 2 x 200 µL of cell-free supernatant are frozen at -80°C. One aliquot of 50 µL of
324 cell-free supernatant is combined with 200 µL of RNA-preserving buffer (RNA later, Qiagen, Germany)
325 and then frozen at -80°C.

326 *Peritoneal fluid:*

327 During surgery, the peritoneal fluid will be collected by the surgeon using a syringe or through
328 mechanical suction on ice. Depending on the volume (up to 15 mL), an aliquot will be centrifuged at
329 300 g, and the pellet (cells) stored at -80°C for further analysis. The cell-free supernatant will also be
330 stored at -80°C.

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

333 All tissue will be collected on ice and divided for storage at -80°C and – after fixing in paraformaldehyde
334 and ethanol – at room temperature. Parts of fresh tissues will be used for culturing experiments, in
335 order to test compounds, drugs or similar agents on primary cells.

336 *Hysterectomy:*

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

342 *Assays*

343 *RNA analysis*

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

348 *Protein analysis*

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

353 *Cells*

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

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

359 *Secretome analysis (perfusion)*

360 Whole uteri with and without fibroids will be perfused with a suitable buffer for up to 8 hours. The
361 perfusate will be analysed by proteomics methods (see above) to detect factors secreted by the
362 fibroids.

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3 363 *Microscopy*

4 364 Tissue blocks (up to 5 cubic millimetres in size) from perfused uteri will be stained with antibodies
5 365 against markers of blood vessels and fibroids, and leakiness, and be recorded in a confocal microscope
6 366 in order to render a three-dimensional image of the blood vessels *in situ*. The detailed study of these
7 367 will allow us to determine whether there is a significant difference in the architecture of blood vessels
8 368 in uteri with fibroids compared to those from uteri without fibroids.

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14 370 **Discontinuation/Withdrawal of Participants from Study**

15 371 Each participant has the right to withdraw from the study at any time. In addition, the Investigator may
16 372 discontinue a participant from the study at any time if the Investigator considers it necessary for any
17 373 reason including:

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20 374 • Pregnancy
21 375 • Ineligibility (either arising during the study or retrospectively, having been missed at screening)
22 376 • Withdrawal of Consent
23 377 • Loss to follow up
24 378 • Loss of mental capacity

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26 379 Withdrawal from the study: At the point the participant withdraws from the study, we will ask for
27 380 consent to retain samples and data collected up to that point.

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29 381 Withdrawn participants will not be replaced.

30 382 The reason for withdrawal will be recorded in the CRF.

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33 383 *Definition of End of Study*

34 384 The end of study is six months after the locking of the study database, to allow for completion of data
35 385 analysis.

36 386 **Patient and Public Involvement**

37 387 FENOX was built on experience and feedback we received from patients and research nurses during a
38 388 previous study (A study to identify possible biomarkers in women with Endometriosis at Oxford –
39 389 ENDOX[18]). In addition, the research objectives were set in accordance with research priorities
40 390 identified through the James Lind Alliance Priority Setting Partnership (PSP) for endometriosis, in which
41 391 we participate[19]. The James Lind Alliance brings patients, carers and clinicians together in Priority
42 392 Setting Partnerships (PSPs) to identify and prioritise the Top 10 unanswered questions or evidence
43 393 uncertainties that they agree are the most important.

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53 394 **INTERVENTIONS**

54 395 **Non-clinical Interventions**

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396 *Questionnaires:*

397 Participants will complete specific questionnaires about their condition, general health, pain sensitivity,
398 medication and menstrual history before their surgery. Additionally, those women with endometriosis
399 will be asked to complete the Endometriosis Health Profile (EHP-30) Questionnaire[20], while women
400 with fibroids complete a section on their quality of life (UFS-QoL[21]). Currently, the clinical
401 questionnaires are completed by participants on paper; in the future, the aim is that the questionnaires
402 can be completed via a handheld device or via the internet directly onto a secure server. For this,
403 women will receive a pre-trial study number and login information. The follow-up questionnaires ask
404 about symptoms and changes in menstrual history as relevant. The control groups would be given the
405 same questionnaires as the women with the respective condition and asked to omit questions not
406 applicable to them.

408 *Medical records:*

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

411 **Clinical Interventions**

412 *Venepuncture:*

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

414 *Collection of other bodily fluid sample:*

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

417 *Tissue collection:*

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

419 *Laparoscopy:*

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

422 **Additional Risk:**

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

424 *Myomectomy/hysterectomy:*

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

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

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3 429 *Trans-cervical resection of fibroids:*

4 430 Endometrial and myometrial biopsies will be taken during surgery.

5 431 Additional Risk: Minor bleeding, uterine perforation (<1%), additional length of surgery by 5 min.

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8 432 *Additionally:*

9 433 In women opting in, an additional endometrial biopsy will be taken during a follow-up visit in an
10 434 outpatient setting. The biopsy of the endometrium is a simple, routine procedure and takes about 30
11 435 minutes.

12 436 Additional risk: Minor bleeding, uterine perforation (<1%), short period of discomfort.

13 437

14 438 Women will be asked to consent to the use of samples and clinical data collected as part of this research
15 439 and in future research. Women, if they consent, will potentially be contacted for future studies
16 440 approved by an ethics committee.

17 441

18 442 **Adverse events**

19 443 For this study, it is conceivable that additional procedures may result in bleeding. However, if this
20 444 resulted in a scenario mentioned below, it would constitute an SAE and needed to be reported to the
21 445 sponsor.

22 446

23 447 A serious adverse event is any untoward medical occurrence that:

- 24 448
- 25 449 • results in death
 - 26 450 • is life-threatening
 - 27 451 • requires inpatient hospitalisation or prolongation of existing hospitalisation
 - 28 452 • results in persistent or significant disability/incapacity
 - 29 453 • consists of a congenital anomaly or birth defect

30 454 Other 'important medical events' may also be considered serious if they jeopardise the participant or
31 455 require an intervention to prevent one of the above consequences.

32 456 NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the
33 457 participant was at risk of death at the time of the event; it does not refer to an event which
34 458 hypothetically might have caused death if it were more severe.

35 459

36 460 A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a
37 461 favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related'
38 462 (resulted from administration of any of the research procedures) and 'unexpected' in relation to those
39 463 procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of
40 464 the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form
41 465 (see HRA website).

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60**466 Data Analysis Plan**

467 As this is a prospective sample and data collection study, there is no randomisation of patients as all
468 women will undergo surgery as part of their routine clinical management. As previously[18], we will
469 use SPSS, Graph Pad Prism, STATA and R for analysis, and employ t-tests, ANOVA, correlation coefficient
470 analysis and similar methods. We plan to use multivariate logistic regression models in comparisons of
471 endometriosis cases with controls, and fibroid cases with controls, adjusting for confounders relevant
472 to the hypothesis being tested. A priori confounders are likely to be age, ethnicity and menstrual cycle
473 phase. Patients with both endometriosis and fibroids will enter into the analysis according to the
474 research question asked; we will conduct sensitivity analyses on this comorbid group to examine to
475 what extent they influence the results. Due to the exploratory nature of this study, various additional
476 statistical techniques may also be used to fully explore the relationships in the data, but all methods
477 will be fully documented.

478 Power Calculations

479 Power calculations were done in R (v3.6.1) using the *pwr* package. For our primary outcome we plan to
480 correlate questionnaire and laboratory data. To detect correlations with a moderate effect size of $r=0.3$
481 at a power of 80% and 0.05 significance level, we will need 85 samples per group (*pwr.r.test*). For the
482 detection of effect sizes of at least 0.2 between groups (e.g. endometriosis cases vs controls, with 3
483 cycle phases and 5 disease stages (0, stages 1 – 4) using ANOVA at 0.05 significance and 80% power, at
484 least 32 samples per groups will be used, with a total of 480 samples for all 15 groups (*pwr.anova.test*).
485 Multiple comparisons will be corrected for by Bonferroni's method.

486 The Number of Participants

487 It is now recognised that both endometriosis and uterine fibroids are very heterogeneous conditions.
488 Our previous studies[22,23] and systematic reviews[24,25] have clearly identified a lack of sufficiently
489 powered studies. Multiple large-scale research collaborations are currently in place investigating
490 different aspects of endometriosis[26], and we plan similar efforts for uterine fibroids. Therefore, large
491 patient numbers are needed.

492 The Endometriosis CaRe Centre at Oxford is the UK's largest endometriosis centre. Similarly, as a
493 tertiary referral centre, we see many women with fibroid-associated symptoms. As a result, we have
494 the unique opportunity to collect large amounts of data and samples, which is essential to produce
495 clinically meaningful outputs. Given our current patient recruitment rate (endometriosis: 100/year,
496 uterine fibroids, 200/year) we estimate an enrolment of approximately 2×1200 women over the
497 course of the study (800 endometriosis patients + 400 non-endometriotic controls, 800 fibroid patients
498 + 400 non-fibroid controls). Fibroids already collected as excess tissue under the Oxford Radcliffe
499 Biobank (ORB, REC Ref 09/H0606/5+5) will also be included in this study, currently approximately 70
500 samples.

501 Analysis of Outcome Measures

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

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3 504 Laboratory data will be analysed using assay-specific software packages employing univariate and
4 505 multivariate pattern recognition methods (e.g. principal component analysis, partial least squares,
5 506 stochastic neighbour embedding algorithms) between sample groups. Correlation with questionnaire
6 507 data will allow us to validate prospective markers of disease. In addition, we will use laboratory data to
7 508 predict disease severity (revised American Fertility Society score[27]), quality of life (EHP-30[20]), pain
8 509 measures and improvement of symptoms as per follow-up questionnaires. For the multivariate
9 510 predictive methods, a test set of approximately 30% of each treatment group will be selected at
10 511 random. This may be selected in a stratified method and exclude patients that have particularly
11 512 extreme values (e.g. > 3 SD from the mean). Patients not included in the test set will make up the
12 513 training set. Models will then be built on the training set and assessed for predictability on the test set.
13 514 The final analysis will be performed on the whole data set. However, if some influential differences e.g.
14 515 in BMI or comorbidities are seen, then e.g. the women with endometriosis will be matched to
15 516 corresponding women without endometriosis, or women with fibroids to women without fibroids, and
16 517 the analysis based on these matched pairs.

518 **DATA MANAGEMENT**

519 **Access to Data**

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

522 **Data Recording and Record Keeping**

523 Each participant will receive a unique study number, which will then be used throughout the study. A
524 study master sheet linking patient identifiable data (name, DOB, hospital and NHS numbers) with the
525 unique study number will be kept and password protected on the University of Oxford's High
526 Compliance server with authorised access and in a file separate from the main study file. Hard copy
527 study documents will be kept in a locked room at each participating centre. Research data will therefore
528 be using non-identifiable data, and all records will be identified only by this study number. All study
529 data will be entered on a desktop computer into a program such as Microsoft EXCEL or Sapphire
530 (Labvantage) using password protection. The participants will be identified by study number in any
531 database. The name and any other identifying details will NOT be included in any electronic file of study
532 data.

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

538 **QUALITY ASSURANCE PROCEDURES**

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3 539 The study may be monitored, or audited in accordance with the current approved protocol, GCP,
4 540 relevant regulations and standard operating procedures.
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8 541 **ETHICAL AND REGULATORY CONSIDERATIONS**

9 542 **Declaration of Helsinki**

10 543 This study will be conducted in accordance with the principles of the Declaration of Helsinki.

11 544 **Guidelines for Good Clinical Practice**

12 545 The Investigator will ensure that this study is conducted in accordance with relevant regulations and
13 546 with Good Clinical Practice.

14 547 **Approvals**

15 548 The protocol, informed consent form, participant information sheet and any proposed advertising
16 549 material will be submitted to an appropriate Research Ethics Committee (REC), and HRA for written
17 550 approval.

18 551 The Investigator will submit and, where necessary, obtain approval from the above parties for all
19 552 substantial amendments to the original approved documents.

20 553 **Reporting**

21 554 The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the
22 555 REC Committee, HRA (where required) host organisation and Sponsor. In addition, an End of Study
23 556 notification and final report will be submitted to the same parties.

24 557 **Participant Confidentiality**

25 558 The study staff will ensure that the participants' anonymity is maintained. The participants will be
26 559 identified only by a participant ID number on all study documents and any electronic database, with
27 560 the exception of the CRF, where participant initials may be added. All documents will be stored securely
28 561 and only accessible by study staff and authorised personnel. The study will comply with the Data
29 562 Protection Act, which requires data to be anonymised as soon as it is practical to do so.

30 563 **Expenses and Benefits**

31 564 There will be no payments made to study participants.

32 565 **Other Ethical Considerations**

33 566 Participants unable to consent for themselves will not be included in the study.

34 567 Patients under clinical management for infertility will be approached in a most sensitive manner by our
35 568 experienced and well-trained team. It is unlikely that our genetic analysis of the participants will reveal
36 569 anything relevant beyond their normal clinical care so we do not plan to report any such findings to
37 570 them or their GPs.

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39 572 **ETHICS AND DISSEMINATION**

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3 573 Approval for this study has been granted by the South Central - Oxford B Research Ethics Committee
4 574 (REC No. 17/SC/0664) on 31st January 2018, by the Health Research Authority (HRA) on 28th February
5 575 2018, and by the Oxford University Hospitals NHS Foundation Trust on 20th March 2018.

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7 576 The findings will be published in high-ranking journals in the field and presented at national and
8 577 international conferences.

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14 579 **DISCUSSION**

15 580 This study has been designed to address both uterine fibroids and endometriosis as conditions that
16 581 both affect the female reproductive system and pose similar problems with regards to pain treatment,
17 582 fertility and quality of life. By combining the patient collectives into one study, we hope to make use of
18 583 synergies between the investigations of the two conditions, in addition to the apparent comorbidity
19 584 between endometriosis patients and those with uterine fibroids [28,29]. Uniquely, the study protocol
20 585 allows for the sampling of endometrium on a follow-up visit, which will allow for the assessment of the
21 586 local, molecular effects of treatment within the same participant.

22 587 FENOX has been designed with the EPHeCT principles [14–17] in mind to ensure standardisation and
23 588 reproducibility, and thus should deliver high-quality datasets that will be useful and comparable
24 589 between centres. We are currently expanding the collection of samples to sites outside of Oxford, with
25 590 a view to make FENOX a multi-centre study within the UK eventually.

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28 593 Oxford, Oxford, UK

29 594 ²Department of Cellular Pathology, Oxford University Hospitals, Oxford, UK

30 595 ³Wellcome Centre for Human Genetics, University of Oxford, Oxford

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32 597 *Acknowledgements*

33 598 We thank all women who participated in FENOX and its predecessor, ENDOX. We acknowledge the
34 599 indispensable help and expertise of Fiona Goddard, NDWRH, and Karen Melham, CTRG, University of
35 600 Oxford, in preparing the study protocol.

36 601 *Contributors*

37 602 TTT wrote the study protocol with KTZ and CMB. CH, KB and ES consented participants and collected
38 603 samples. HMN, KSS and TTT processed samples. KG, EIJ and CC documented samples and designed
39 604 electronic questionnaires. SM determined menstrual cycle stages by histology. KTZ and CMB conceived
40 605 the study.

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611 & Reproductive Health under the Oxford/Bayer-Alliance for Women's Health.

612 *Competing interests*

613 CMB and KTZ received research grants from Bayer Healthcare, Volition RX, MDNA Life Sciences and
614 Roche Diagnostics. The study is funded by the Nuffield Department of Women's & Reproductive Health.

615 *Patient consent*

616 See above, *informed consent*

617 *Ethics approval*

618 Approval has been granted by the South Central - Oxford B Research Ethics Committee (REC No.
619 17/SC/0664), by the Health Research Authority (HRA), and by the Oxford University Hospitals NHS
620 Foundation Trust.

621 *Provenance and peer review*

622 The FENOX protocol was based upon the previous ENDOX study (REC reference 09/H0604/58). It was
623 originally approved in January 2018, with amendments approved in April 2019.

624 *Open access*

625 The study protocol will be published under a Creative Commons licence.

626 *Data sharing*

627 The authors will make relevant anonymised patient-level data available upon reasonable request,
628 according to the established standards in the field. Data that could compromise participant anonymity
629 or privacy will not be shared in any way.

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FIGURE LEGENDS

Figure 1: Participant groups and procedures. The participants will be allocated into case or control groups and tissues will be sourced according to the course of clinical intervention: Participants with fibroids treated by transcervical resection (TCRF) will not undergo abdominal surgery, while abdominal surgery is necessary if the fibroids are treated by laparoscopy, open myomectomy or hysterectomy. In these cases, peritoneal fluid can be obtained in addition to the tissue samples (fibroid, myometrium, endometrium). Endometriosis patients will undergo laparoscopy. If found without endometriosis, they will be grouped with the controls, who are women undergoing surgery for other, unrelated conditions (e.g. tubular ligation).

Figure 2: Flowchart of participant data and tissue sampling. Consented participants of the different arms of the study donate pre-operative samples and fill in a baseline questionnaire. They can donate a range of tissue samples according to their condition and treatment mode (e.g. fibroids or endometriotic tissue, peritoneal fluid), and are asked to repeatedly fill in follow-up questionnaires on their condition and quality of life, so that the clinical findings can be correlated with the outcome years later.

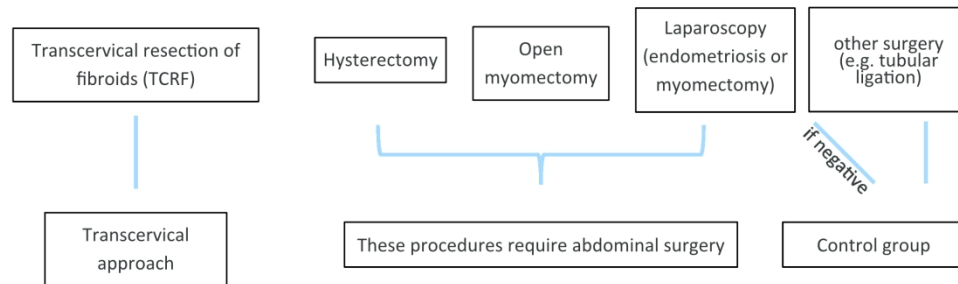


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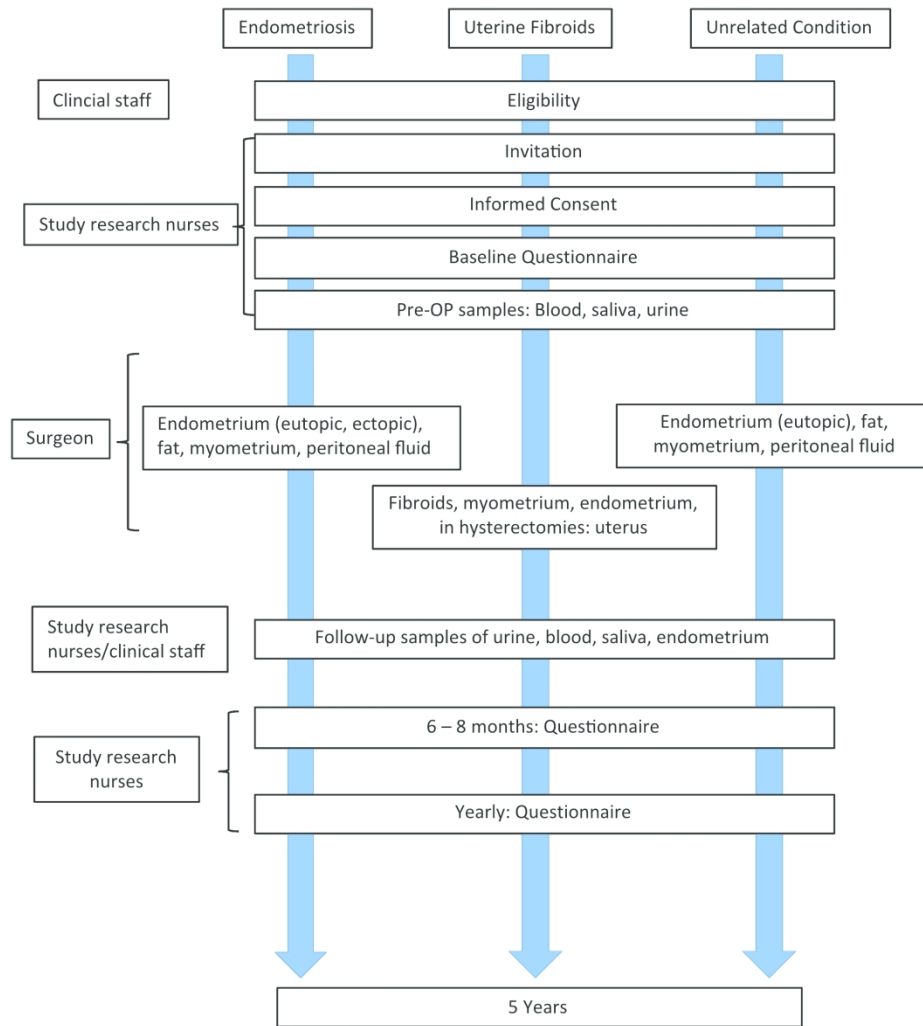


Figure 2: Flowchart of participant data and tissue sampling. Consented participants of the different arms of the study donate pre-operative samples and fill in a baseline questionnaire. They can donate a range of tissue samples according to their condition and treatment mode (e.g. fibroids or endometriotic tissue, peritoneal fluid), and are asked to repeatedly fill in follow-up questionnaires on their condition and quality of life, so that the clinical findings can be correlated with the outcome years later.

BMJ Open

A protocol for a longitudinal, prospective cohort study investigating the biology of uterine fibroids and endometriosis, and patients' quality of life – the FENOX study.

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Date Submitted by the Author:	27-Jan-2020
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5 2 Title: **A protocol for a longitudinal, prospective cohort study investigating the biology of uterine**
6
7 3 **fibroids and endometriosis, and patients' quality of life – the FENOX study.**

8
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24
25 12 **ABSTRACT**

26
27 13 **Introduction**

28
29 14 Millions of women suffer from the consequences of endometriosis and uterine fibroids, with fibroids
30
31 15 the cause for over 50% of hysterectomies in the US, and direct costs for their treatment estimated at
32
33 16 between 4 and 9 billion USD. Endometriosis commonly affects millions of women worldwide
34
35 17 predominantly during reproductive age, with severe menstrual and non-menstrual pain and subfertility
36
37 18 the main symptoms. Due to the 'unhappy triad' of endometriosis – lack of awareness, lack of clinically
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39 19 relevant biomarkers and the unspecific nature of symptoms – women wait on average for 8–12 years
40
41 20 before the definitive endometriosis diagnosis is made. Treatment options for both conditions are not
42
43 21 satisfactory at the moment, especially with a view to preserving fertility for the women and families
44
45 22 affected. In the Fibroids and Endometriosis Oxford (FENOX) study, we combine the investigation of
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47 23 fibroids and endometriosis, and plan to collect high quality tissue samples and medical data of
48
49 24 participants over a time frame of 5 years after surgical intervention.

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51 25
52 26 **Methods and analysis**

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54 27 Biological samples such as blood, saliva, urine, fat, peritoneal fluid and – if found – endometrial tissue
55
56 28 or fibroids as well as detailed clinical and intraoperative data will be collected from women undergoing
57
58 29 surgery and participating in the study after informed consent. We plan to recruit up to 1200 participants
59
60 30 per disease arm (i.e. endometriosis and uterine fibroids) over 5 years. Participants will fill in detailed
31
32 31 and validated questionnaires on their medical history and quality of life, with follow-ups for 5 years.
33
34 32 Enrolment started on 2nd April 2018, and FENOX will close on 31st March 2028. We will analyse the
35
36 33 biological samples using state-of-the-art molecular biology methods and correlate the findings with the
37
38 34 medical records and questionnaire data.

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5 36 **Ethics and dissemination**6
7 37 Approval for this study has been granted by the South Central - Oxford B Research Ethics Committee
8 38 (REC No. 17/SC/0664), by the Health Research Authority (HRA), and by the Oxford University Hospitals
9
10 39 NHS Foundation Trust. The findings will be published in high-ranking journals in the field and presented
11 40 at national and international conferences.
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14 42 **Trial registration number**15
16 43 ISRCTN13560263; <https://doi.org/10.1186/ISRCTN13560263>
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For peer review only

ARTICLE SUMMARY

Strengths and limitations of this study

- FENOX combines the study of endometriosis and uterine fibroids to identify the underlying mechanisms of both conditions.
- The study comprises biological samples as well as comprehensive phenotypic data.
- World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonisation Project (EPHect) criteria are applied to ensure the best possible standardisation.
- The longitudinal aspect is an important feature of FENOX but this depends on uptake and compliance by participants.
- The control group comprises of women undergoing surgery for gynaecological indications other than endometriosis or uterine fibroids; thus, they are not completely healthy controls.

INTRODUCTION

Millions of women suffer from the consequences of endometriosis [1,2] and uterine fibroids [3,4]. These include pelvic and abdominal pain, abnormal uterine bleeding, infertility and miscarriages [5–8]. 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 [9], and direct costs for their treatment is estimated between 4 and 9 billion USD [10].

Clinically relevant, non-invasive diagnostic tests including biomarkers or imaging techniques do not exist for many forms of endometriosis [11–13] 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, 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) [14–17], and we are planning to establish similar standards in uterine fibroid research.

In the FENOX 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

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82 women undergoing surgery. These samples will be used in state-of-the-art biomedical assays (see
83 'Assays') to improve our understanding of the underlying biology of these symptoms in women with
84 endometriosis and/or fibroids, which will lead to a better understanding of the conditions, stratification
85 of patient groups and tailored therapies, and the development of novel drug targets and biomarkers
86 for diagnosis and treatment.

87 88 **Objectives**

89 *Primary objective*

- 90 • To identify the underlying mechanisms of endometriosis and uterine fibroids and their
91 associated symptoms to improve the outcome of affected women.

92 93 *Secondary objectives*

- 94 • To identify novel biomarkers of endometriosis.
- 95 • To identify clinical subgroups of endometriosis and uterine fibroids.
- 96 • To understand the genetics underlying these conditions and explore the relevant downstream
97 molecular pathways.
- 98 • To investigate the relation between the presence of fibroids and the symptoms, e.g. abnormal
99 uterine bleeding.
- 100 • To identify novel drug targets.
- 101 • To develop models of disease progression and prediction.
- 102 • To investigate conditions or symptoms associated with endometriosis and/or uterine fibroids,
103 including: symptoms and characteristics of the female reproductive system (characteristics of
104 menstrual bleeding, fertility, infertility, pregnancy outcomes), pelvic as well as non-pelvic pain
105 conditions, metabolic phenotypes (polycystic ovarian syndrome (PCOS), obesity and fat
106 distribution), cardiovascular conditions and symptoms, neuroangiogenesis and related
107 neurological symptoms, immunological disorders, and cancers.

108 109 **Outcomes**

110 *Primary outcome*

111 We will use questionnaire data, medical records and sample analysis to investigate the genetic and
112 molecular basis of the pathogenesis and symptoms of endometriosis and uterine fibroids. At the end
113 of the recruiting period, i.e. from December 2022 onwards, the collected data and samples will be
114 analysed and compared between endometriosis/fibroid cases, and non-affected controls.

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115 *Secondary outcomes*

116 Prospective standardised questionnaires and samples will be collected according to EPHeC standards.

117 The correlation of cellular, molecular and genetic data and endometriosis status will allow us to define
118 novel biomarkers of the disease.

119 Clinical notes and questionnaires in combination with sample data will be used to define clinical
120 subgroups of patients.

121 The molecular and genetic findings will be compared against public databases of disease-relevant
122 molecular pathways, and *in vitro* experiments will be carried out to test hypothetical connections
123 between the genetics and manifestation of disease.

124 The blood vessels and endothelial cells will be compared between tissue from women presenting with
125 fibroids and those without.

126 The detailed comparison between tissue from women with fibroids and those without will yield
127 differences in terms of proteins expressed; these can then be tested as targets using known or new
128 drugs.

129 As data accumulate and genetic mechanisms become clear, hypotheses will be formed as to the likely
130 progression of disease. These will be tested against the reports from the follow-up questionnaires.

131 We will use questionnaire data, medical records and sample analysis to investigate the genetic and
132 molecular basis of the pathogenesis and symptoms of conditions or symptoms associated with
133 endometriosis and/or uterine fibroids.

134 **METHODS AND ANALYSIS**

135 **Study design**

136 FENOX is a prospective study that aims to improve our understanding of the underlying mechanisms of
137 endometriosis and uterine fibroids and their associated symptoms by means of longitudinal
138 observation and laboratory analyses. Biological samples such as blood, saliva, urine, fat, peritoneal
139 fluid and – if found - endometriosis tissue or fibroids as well as detailed clinical and intraoperative data
140 will be collected from women of reproductive age with and without endometriosis- and fibroid-
141 associated symptoms, such as pain, abnormal uterine bleeding and infertility. Women undergoing
142 surgery for these conditions, and women undergoing surgery for unrelated gynaecological conditions
143 as part of their normal clinical management will be asked to participate (Fig. 1). An incidental diagnosis
144 of endometriosis or uterine fibroids will lead to the patients' inclusion into the relevant case groups. All
145 women attending clinics receive a letter informing them of ongoing research, and eligible women will
146 be identified initially by research nurses or clinical staff during clinic visits. Once a woman has expressed
147 an interest in participating in this study, they will be consented by a member of the research team
148 (flowchart, Fig. 2).

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

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3 151 In order to determine the effect of the surgical removal of the fibroids on the local tissue, it is necessary
4 152 to take an additional endometrial biopsy after the planned surgical intervention. This sample will be
5 153 timed to synch with the same time point in the menstrual cycle that the original sample was taken, and
6 154 thus will give us a unique insight into the biology of the conditions. During this visit, blood and urine
7 155 samples will be taken again also. Women can opt in or out of the additional clinic visit where these
8 156 samples would be taken.

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14 158 Women will be asked to complete questionnaires on paper, online or into their electronic handheld
15 159 devices (health, pain, medication and, initially, ethnicity) at different time points. There will be a lengthy
16 160 questionnaire at baseline before surgery (taking an estimated 45 minutes to complete), and shorter
17 161 versions (taking up to 30 minutes to complete) post-operatively at 6-8 weeks, 6 months, 12 months and
18 162 thereafter yearly for a total of five years after surgical intervention.

19 163 **Samples**

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23 164 A. Blood samples (up to 50 ml, venepuncture), urine (micturition) and saliva (spit) will be taken
24 165 prior to surgery.

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26 166 B. During surgery, tissue samples will be taken as specified below.

27 167 C. In women opting in, an additional endometrial biopsy will be taken during a follow-up visit at
28 168 least three months after surgery. This can be done in an outpatient setting, and the taking of
29 169 an endometrial biopsy in this setting using an endometrial sampling device (e.g. pipelle or
30 170 curette) is an established technique. A blood and urine sample will be taken again also.

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35 172 **Participants**

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37 173 In each of the disease arms (endometriosis or fibroids), we plan to recruit up to 1200 women of
38 174 reproductive age (18 years until menopause) who are planned to undergo surgery. 800 of these will
39 175 have the condition of interest, and 400 will be having surgery for other reasons and act as controls. In
40 176 addition, we will include fibroid and uterine tissue samples collected as excess tissue (Oxford Radcliffe
41 177 Biobank, REC Ref 09/H0606/5+5), currently approximately 70 samples.

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45 179 **Inclusion criteria**

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- 48 181 • The participant is willing and able to give informed consent for participation in the study.
 - 49 182 • The participant is female and aged 18 years or above (before menopause).
 - 50 183 • Women undergoing planned surgery (including hysterectomy) for endometriosis- and/or
 - 51 184 fibroid associated symptoms such as abdominal pain, abnormal uterine bleeding, or for
 - 52 185 unrelated gynaecological conditions (e.g. fertility investigation or for laparoscopic tubal
 - 53 186 sterilisation).

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57 187 **Exclusion criteria**

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59 188 The participant may not enter the study if ANY of the following apply:

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

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193 **Participant enrolment**

194 *Recruitment*

195 After general information through a generic letter with information about ongoing research, which
196 every patient will receive prior to her outpatient appointment, eligible women will be identified initially
197 by the research nurses or clinical team during clinic visits. The study research nurses will then contact
198 those women interested in participating in the study.

199 *Screening and Eligibility Assessment*

200 Women attending clinic appointments for endometriosis- and fibroid-associated symptoms such as
201 pain, abnormal uterine bleeding, and infertility will be asked to participate by clinical staff or by the
202 authorised study research nurses. Women undergoing surgery for these conditions, and women
203 undergoing surgery as part of their normal clinical management (e.g. laparoscopic tubal ligation or
204 hysterectomy; they would be the control patients) are eligible to participate in the study.

205 *Informed Consent*

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

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

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

218 Written informed consent will then be obtained by means of the participant's dated signature and the
219 dated signature of the person who presented and obtained the informed consent. The person who
220 obtained the consent will be suitably qualified and experienced, and will have been authorised to do
221 so by the Chief Investigator. A copy of the signed informed consent and the patient information sheet
222 will be given to the participant. The original signed form will be retained at the study site. The consent
223 form for this study allows for the participant declining consent for any procedure that she is not
224 comfortable with, while remaining eligible as a participant of the study. E.g. if a participant did not want

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3 225 a uterine biopsy used in the study, she would not initial the corresponding box on the consent form
4 226 and insert 'No' instead.

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8 228 **Study settings**

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10 229 *Baseline Assessments*

11 230 Consented participants will be asked by the research team to complete a baseline questionnaire before
12 231 their surgery. This may be sent to them e.g. via mail or e-mail before surgery or given to them in paper
13 232 form in the clinic. Alternatively, participants have the option to complete an online version of the
14 233 questionnaire or they may fill in the questionnaire on their hand-held device. Each participant will
15 234 receive a unique login for the online questionnaires.

16 235 The questionnaire data will be withheld from the research team until written informed consent is
17 236 obtained and will be destroyed if this is not granted.

18 237 All participants will be sent further questionnaires at different time points (approximately 6-8 weeks, 6
19 238 months, 12 months and then yearly for 5 years after surgery). Participants may be reminded (twice,
20 239 maximally) to return completed questionnaires via mail, email, phone or text or similar.

21 240 On the day of surgery, they will be asked to provide a mid-stream urine and a saliva sample. In addition,
22 241 blood will be collected by peripheral venepuncture. The procedures will be explained to the women
23 242 again and they will be given the opportunity to ask questions. Assessment of the presence and extent
24 243 of disease will be performed by the operating surgeon.

25 244 *Samples that may be taken at time of surgical procedures are as follows:*

26 245

27 246 1. *Laparoscopy for suspected endometriosis:*

28 247 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue if present,
29 248 peritoneal biopsy.

30 249

31 250 2. *Laparoscopy for uterine fibroids:*

32 251 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue (if present),
33 252 peritoneal biopsy, fibroid tissue, myometrial biopsy.

34 253

35 254 3. *Laparotomy for uterine fibroids:*

36 255 Endometrial biopsy, peritoneal fluid aspiration, fat tissue biopsy, endometriotic tissue if present,
37 256 peritoneal biopsy, fibroid tissue, myometrial biopsy.

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39 258 4. *Trans-cervical resection of uterine fibroids (TCRF):*

40 259 Endometrial biopsy, fibroid tissue, myometrial biopsy

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261 5. *Laparoscopy for tubal sterilisation (Controls):*

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

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264 6. *Hysterectomy:*

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

268

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

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

277

278 *Subsequent Visits*

279 Unless participants underwent a hysterectomy, all included women will be asked to contact the study
280 team when the next menstrual period after the procedure started. Together with the last menstrual
281 period (LMP) date given at the time of the procedure, this date will be used to calculate the length of
282 the cycle. This is important in order to account for the changes that occur in the uterus during the
283 menstrual cycle, and to enable us to distinguish between the effects of the cycle and the disease.

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

291 All women will be contacted by a member of the medical or study team and asked to fill in further
292 questionnaires at different follow-up time points (approximately 6-8 weeks, 6 months, 12 months and
293 yearly thereafter for a total of 5 years). Reminders will be sent twice, maximally. Women will also be
294 asked if they can be contacted in the future for any further studies approved by an ethics committee.

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5 296 **Sample Handling**

6 297 Samples will be obtained according to WERF/EPHect guidelines[14–17] by the interventions listed
7
8 298 below. Only the study team will have access. Biological samples will be stored at -20°C or at -80°C for
9
10 299 use in current and future studies until exhausted. Disease and control samples will be stored under the
11
12 300 same conditions. For the purposes of this study, samples may be analysed at Oxford, or they may be
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14 301 transferred to a third party/study collaborator, including industrial partners, for analysis at their facility.
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16 302 If participants agree, samples will be moved to a Research Tissue Bank at the end of the study or stored
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18 303 and used in future ethically approved studies. They would be made available in anonymised form.

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20 304 To investigate the relationship between uterine fibroids and symptoms such as abnormal uterine
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22 305 bleeding and pain, we aim to collect endometrial and myometrial samples alongside the fibroids
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24 306 themselves, to be able to detect the effects of fibroids on their surroundings.

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26 307 We also intend to use the endometrium and endometriotic samples, one of the blood samples and fat
27
28 308 samples to look for genetic factors and molecular pathways that can lead to endometriosis or uterine
29
30 309 fibroids. The samples for this analysis will also be anonymised so that we do not know specifically which
31
32 310 patient they came from. However, all samples are identifiable with printed label and location detail,
33
34 311 participant ID, sample type and colour-coded cap.

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37 312 *Blood:*

38
39 313 50 mL. These are divided into (at least) EDTA- (2 x 9 mL) and heparin-treated samples (2 x 6 mL, both
40
41 314 anti-coagulation), serum (2 x SST, 5 mL) and two plain blood samples of 5 mL. The different vials are
42
43 315 colour-coded and frozen at -80°C.

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46 316 *Urine:*

47
48 317 20 mL. Half of the sample will be used to test for glucose by specific gravity assay, the other half will be
49
50 318 stored for the study. One aliquot of 5 mL is frozen directly at -80°C; 1 mL is centrifuged at 300 g, and 5
51
52 319 aliquots a 200 µL of cell-free supernatant are frozen at -80°C.

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55 320 *Saliva:*

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57 321 A spit sample of approximately 1 mL is taken on ice. One aliquot of 200 µL is frozen at -80°C, the rest is
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59 322 centrifuged at 300 g and 2 x 200 µL of cell-free supernatant are frozen at -80°C. One aliquot of 50 µL of
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323 cell-free supernatant is combined with 200 µL of RNA-preserving buffer (RNA later, Qiagen, Germany)
324 and then frozen at -80°C.

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327 325 *Peritoneal fluid:*

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329 326 During surgery, the peritoneal fluid will be collected by the surgeon using a syringe or through
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331 327 mechanical suction on ice. Depending on the volume (up to 15 mL), an aliquot will be centrifuged at
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333 328 300 g, and the pellet (cells) stored at -80°C for further analysis. The cell-free supernatant will also be
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335 329 stored at -80°C.

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

332 All tissue will be collected on ice and divided for storage at -80°C and – after fixing in paraformaldehyde
333 and ethanol – at room temperature. Parts of fresh tissues will be used for culturing experiments, in
334 order to test compounds, drugs or similar agents on primary cells.

335 *Hysterectomy:*

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

341 *Assays*

342 *RNA analysis*

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

347 *Protein analysis*

348 Proteins will be extracted from tissue samples using standard methods. The expression and amount of
349 proteins will be analysed by immunoblotting for specific proteins of interest, and by proteomics
350 methods using the matrix-assisted laser desorption/ionization (MALDI)/surface-enhanced laser
351 desorption/ionization (SELDI) platform. Tissue sections will be used in standard immunohistochemistry
352 to detect the expression of markers of interest *in situ*.

353 *Cells*

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

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

359 *Secretome analysis (perfusion)*

360 Whole uteri with and without fibroids will be perfused with a suitable buffer for up to 8 hours. The
361 perfusate will be analysed by proteomics methods (see above) to detect factors secreted by the
362 fibroids.

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3 363 *Microscopy*

4 364 Tissue blocks (up to 5 cubic millimetres in size) from perfused uteri will be stained with antibodies
5 365 against markers of blood vessels and fibroids, and leakiness, and be recorded in a confocal microscope
6 366 in order to render a three-dimensional image of the blood vessels *in situ*. The detailed study of these
7 367 will allow us to determine whether there is a significant difference in the architecture of blood vessels
8 368 in uteri with fibroids compared to those from uteri without fibroids.

9 369

10 370 **Discontinuation/Withdrawal of Participants from Study**

11 371 Each participant has the right to withdraw from the study at any time. In addition, the Investigator may
12 372 discontinue a participant from the study at any time if the Investigator considers it necessary for any
13 373 reason including:

- 14 374
- 15 375 • Pregnancy
 - 16 376 • Ineligibility (either arising during the study or retrospectively, having been missed at screening)
 - 17 377 • Withdrawal of Consent
 - 18 378 • Loss to follow up
 - 19 379 • Loss of mental capacity

20 380 Withdrawal from the study: At the point the participant withdraws from the study, we will ask for
21 381 consent to retain samples and data collected up to that point. Withdrawn participants will not be
22 382 replaced. The reason for withdrawal will be recorded in the case record file (CRF).

23 383 *Definition of End of Study*

24 384 The end of study is six months after the locking of the study database, to allow for completion of data
25 385 analysis.

26 386 **Patient and Public Involvement**

27 387 FENOX was built on experience and feedback we received from patients and research nurses during a
28 388 previous study (A study to identify possible biomarkers in women with Endometriosis at Oxford –
29 389 ENDOX[18]). In addition, the research objectives were set in accordance with research priorities
30 390 identified through the James Lind Alliance Priority Setting Partnership (PSP) for endometriosis, in which
31 391 we participate[19]. The James Lind Alliance brings patients, carers and clinicians together in Priority
32 392 Setting Partnerships (PSPs) to identify and prioritise the Top 10 unanswered questions or evidence
33 393 uncertainties that they agree are the most important.

34 394 **INTERVENTIONS**

35 395 **Non-clinical Interventions**

36 396 *Questionnaires:*

37 397 Participants will complete specific questionnaires about their condition, general health, pain sensitivity,
38 medication and menstrual history before their surgery. Additionally, those women with endometriosis

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398 will be asked to complete the Endometriosis Health Profile (EHP-30) Questionnaire[20], while women
399 with fibroids complete a section on their quality of life (UFS-QoL[21]). Currently, the clinical
400 questionnaires are completed by participants on paper; in the future, the aim is that the questionnaires
401 can be completed via a handheld device or via the internet directly onto a secure server. For this,
402 women will receive a pre-trial study number and login information. The follow-up questionnaires ask
403 about symptoms and changes in menstrual history as relevant. The control groups would be given the
404 same questionnaires as the women with the respective condition and asked to omit questions not
405 applicable to them.

406

407 *Medical records:*

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

410 **Clinical Interventions**

411 *Venepuncture:*

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

413 *Collection of other bodily fluid sample:*

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

416 *Tissue collection:*

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

418 *Laparoscopy:*

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

421 **Additional Risk:**

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

423 *Myomectomy/hysterectomy:*

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

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

428 *Trans-cervical resection of fibroids:*

429 Endometrial and myometrial biopsies will be taken during surgery.

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

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3 431 *Additionally:*

4 432 In women opting in, an additional endometrial biopsy will be taken during a follow-up visit in an
5 433 outpatient setting. The biopsy of the endometrium is a simple, routine procedure and takes about 30
6 434 minutes.

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9 435 Additional risk: Minor bleeding, uterine perforation (<1%), short period of discomfort.

10 436

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12 437 Women will be asked to consent to the use of samples and clinical data collected as part of this research
13 438 and in future research. Women, if they consent, will potentially be contacted for future studies
14 439 approved by an ethics committee.

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18 441 **Adverse events**

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20 442 For this study, it is conceivable that additional procedures may result in bleeding. However, if this
21 443 resulted in a scenario mentioned below, it would constitute an SAE and needed to be reported to the
22 444 sponsor.

23 445

24 446 A serious adverse event is any untoward medical occurrence that:

25 447

- 26 448 • results in death
- 27 449 • is life-threatening
- 28 450 • requires inpatient hospitalisation or prolongation of existing hospitalisation
- 29 451 • results in persistent or significant disability/incapacity
- 30 452 • consists of a congenital anomaly or birth defect

31 453 Other 'important medical events' may also be considered serious if they jeopardise the participant or
32 454 require an intervention to prevent one of the above consequences.

33 455 NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the
34 456 participant was at risk of death at the time of the event; it does not refer to an event which
35 457 hypothetically might have caused death if it were more severe.

36 458

37 459 A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a
38 460 favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related'
39 461 (resulted from administration of any of the research procedures) and 'unexpected' in relation to those
40 462 procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of
41 463 the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form
42 464 (see HRA website).

43 465

44 466 **Data Analysis Plan**

45 467 As this is a prospective sample and data collection study, there is no randomisation of patients as all
46 468 women will undergo surgery as part of their routine clinical management. As previously[18], we will
47 use SPSS, Graph Pad Prism, STATA and R for analysis, and employ t-tests, ANOVA, correlation coefficient

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3 469 analysis and similar methods. We plan to use multivariate logistic regression models in comparisons of
4 470 endometriosis cases with controls, and fibroid cases with controls, adjusting for confounders relevant
5 471 to the hypothesis being tested. A priori confounders are likely to be age, ethnicity and menstrual cycle
6 472 phase. Patients with both endometriosis and fibroids will enter into the analysis according to the
7 473 research question asked; we will conduct sensitivity analyses on this comorbid group to examine to
8 474 what extent they influence the results. Due to the exploratory nature of this study, various additional
9 475 statistical techniques may also be used to fully explore the relationships in the data, but all methods
10 476 will be fully documented.

15 477 **Power Calculations**

16 478 Power calculations were done in R (v3.6.1) using the *pwr* package. For our primary outcome we plan to
17 479 correlate questionnaire and laboratory data. To detect correlations with a moderate effect size of $r=0.3$
18 480 at a power of 80% and 0.05 significance level, we will need 85 samples per group (*pwr.r.test*). For the
19 481 detection of effect sizes of at least 0.2 between groups (e.g. endometriosis cases vs controls, with 3
20 482 cycle phases and 5 disease stages (0, stages 1 – 4) using ANOVA at 0.05 significance and 80% power, at
21 483 least 32 samples per groups will be used, with a total of 480 samples for all 15 groups (*pwr.anova.test*).
22 484 Multiple comparisons will be corrected for by Bonferroni's method.

27 485 **The Number of Participants**

28 486 It is now recognised that both endometriosis and uterine fibroids are very heterogeneous conditions.
29 487 Our previous studies[22,23] and systematic reviews[24,25] have clearly identified a lack of sufficiently
30 488 powered studies. Multiple large-scale research collaborations are currently in place investigating
31 489 different aspects of endometriosis[26], and we plan similar efforts for uterine fibroids. Therefore, large
32 490 patient numbers are needed.

33 491 The Endometriosis CaRe Centre at Oxford is the UK's largest endometriosis centre. Similarly, as a
34 492 tertiary referral centre, we see many women with fibroid-associated symptoms. As a result, we have
35 493 the unique opportunity to collect large amounts of data and samples, which is essential to produce
36 494 clinically meaningful outputs. Given our current patient recruitment rate (endometriosis: 100/year,
37 495 uterine fibroids, 200/year) we estimate an enrolment of approximately 2×1200 women over the
38 496 course of the study (800 endometriosis patients + 400 non-endometriotic controls, 800 fibroid patients
39 497 + 400 non-fibroid controls). Fibroids already collected as excess tissue under the Oxford Radcliffe
40 498 Biobank (ORB, REC Ref 09/H0606/5+5) will also be included in this study, currently approximately 70
41 499 samples.

50 500 **Analysis of Outcome Measures**

51 501 All samples excluding those from patients who withdraw consent will be included in the analysis of
52 502 outcome measures.

53 503 Laboratory data will be analysed using assay-specific software packages employing univariate and
54 504 multivariate pattern recognition methods (e.g. principal component analysis, partial least squares,
55 505 stochastic neighbour embedding algorithms) between sample groups. Correlation with questionnaire
56 506 data will allow us to validate prospective markers of disease. In addition, we will use laboratory data to

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3 507 predict disease severity (revised American Fertility Society score[27]), quality of life (EHP-30[20]), pain
4 508 measures and improvement of symptoms as per follow-up questionnaires. For the multivariate
5 509 predictive methods, a test set of approximately 30% of each treatment group will be selected at
6 510 random. This may be selected in a stratified method and exclude patients that have particularly
7 511 extreme values (e.g. > 3 SD from the mean). Patients not included in the test set will make up the
8 512 training set. Models will then be built on the training set and assessed for predictability on the test set.
9 513 The final analysis will be performed on the whole data set. However, if some influential differences e.g.
10 514 in BMI or comorbidities are seen, then e.g. the women with endometriosis will be matched to
11 515 corresponding women without endometriosis, or women with fibroids to women without fibroids, and
12 516 the analysis based on these matched pairs.

517 **DATA MANAGEMENT**

518 **Access to Data**

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

521 **Data Recording and Record Keeping**

522 Each participant will receive a unique study number, which will then be used throughout the study. A
523 study master sheet linking patient identifiable data (name, DOB, hospital and NHS numbers) with the
524 unique study number will be kept and password protected on the University of Oxford's High
525 Compliance server with authorised access and in a file separate from the main study file. Hard copy
526 study documents will be kept in a locked room at each participating centre. Research data will therefore
527 be using non-identifiable data, and all records will be identified only by this study number. All study
528 data will be entered on a desktop computer into a program such as Microsoft EXCEL or Sapphire
529 (Labvantage) using password protection. The participants will be identified by study number in any
530 database. The name and any other identifying details will NOT be included in any electronic file of study
531 data.

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

537 **QUALITY ASSURANCE PROCEDURES**

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

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60**541 ETHICS AND DISSEMINATION****542 Declaration of Helsinki**

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

544 Guidelines for Good Clinical Practice

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

547 Approvals

548 Approval for this study has been granted by the South Central - Oxford B Research Ethics Committee
549 (REC No. 17/SC/0664) on 31st January 2018, by the Health Research Authority (HRA) on 28th February
550 2018, and by the Oxford University Hospitals NHS Foundation Trust on 20th March 2018.

551 Reporting

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

555 Publications

556 The findings from the study will be published in high-ranking journals in the field and presented at
557 national and international conferences.

558 Participant Confidentiality

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

564 Expenses and Benefits

565 There will be no payments made to study participants.

566 Other Ethical Considerations

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

568 Patients under clinical management for infertility will be approached in a most sensitive manner by our
569 experienced and well-trained team. It is unlikely that our genetic analysis of the participants will reveal
570 anything relevant beyond their normal clinical care so we do not plan to report any such findings to
571 them or their GPs.

572 DISCUSSION

573 This study has been designed to address both uterine fibroids and endometriosis as conditions that
574 both affect the female reproductive system and pose similar problems with regards to pain treatment,
575 fertility and quality of life. By combining the patient collectives into one study, we hope to make use of
576 synergies between the investigations of the two conditions, in addition to the apparent comorbidity
577 between endometriosis patients and those with uterine fibroids [28,29]. Uniquely, the study protocol

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3 578 allows for the sampling of endometrium on a follow-up visit, which will allow for the assessment of the
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5 579 local, molecular effects of treatment within the same participant.
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7 580 FENOX has been designed with the EPHect principles [14–17] in mind to ensure standardisation and
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9 581 reproducibility, and thus should deliver high-quality datasets that will be useful and comparable
10
11 582 between centres. We are currently expanding the collection of samples to sites outside of Oxford, with
12
13 583 a view to make FENOX a multi-centre study within the UK eventually.

13 584 *Author affiliations*

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21 589

22 23 590 *Acknowledgements*

24 591 We thank all women who participated in FENOX and its predecessor, ENDOX. We acknowledge the
25
26 592 indispensable help and expertise of Fiona Goddard, NDWRH, and Karen Melham, CTRG, University of
27
28 593 Oxford, in preparing the study protocol.

29 30 594 *Contributors*

31 595 TTT wrote the study protocol with KTZ and CMB. CH, KB and ES consented participants and collected
32
33 596 samples. HMN, KSS and TTT processed samples. KG, EJJ and CC documented samples and designed
34
35 597 electronic questionnaires. SM determined menstrual cycle stages by histology. KTZ and CMB conceived
36
37 598 the study.

38 39 599 *Funding*

40 600 TTT received funding from the Nuffield Benefaction for Medicine and the Wellcome Institutional
41
42 601 Strategic Support Fund (ISSF, ref no. 5258). HMN received funding from the Oxfordshire Health Services
43
44 602 Research Committee. KG was supported by a grant from the National Institutes of Health USA
45
46 603 (R01HD094842). Funding for this study has been obtained from the Nuffield Department of Women's
47
48 604 & Reproductive Health under the Oxford/Bayer-Alliance for Women's Health.

48 49 605 *Competing interests*

50 606 CMB and KTZ received research grants from Bayer Healthcare, Volition RX, MDNA Life Sciences and
51
52 607 Roche Diagnostics. The study is funded by the Nuffield Department of Women's & Reproductive Health.

53 54 608 *Patient consent*

55 609 See above, *informed consent*
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610 *Ethics approval*

611 Approval has been granted by the South Central - Oxford B Research Ethics Committee (REC No.
612 17/SC/0664), by the Health Research Authority (HRA), and by the Oxford University Hospitals NHS
613 Foundation Trust.

614 *Provenance and peer review*

615 The FENOX protocol was based upon the previous ENDOX study (REC reference 09/H0604/58). It was
616 originally approved in January 2018, with amendments approved in April 2019.

617 *Open access*

618 The study protocol will be published under a Creative Commons licence.

619 *Data sharing*

620 The authors will make relevant anonymised patient-level data available upon reasonable request,
621 according to the established standards in the field. Data that could compromise participant anonymity
622 or privacy will not be shared in any way.

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706 **FIGURE LEGENDS**

707 **Figure 1: Participant groups and procedures.** The participants will be allocated into case or control
708 groups and tissues will be sourced according to the course of clinical intervention: Participants with
709 fibroids treated by transcervical resection (TCRF) will not undergo abdominal surgery, while abdominal
710 surgery is necessary if the fibroids are treated by laparoscopy, open myomectomy or hysterectomy. In
711 these cases, peritoneal fluid can be obtained in addition to the tissue samples (fibroid, myometrium,
712 endometrium). Endometriosis patients will undergo laparoscopy. If found without endometriosis, they
713 will be grouped with the controls, who are women undergoing surgery for other, unrelated conditions
714 (e.g. tubular ligation).

715

716 **Figure 2: Flowchart of participant data and tissue sampling.** Consented participants of the different
717 arms of the study donate pre-operative samples and fill in a baseline questionnaire. They can donate a
718 range of tissue samples according to their condition and treatment mode (e.g. fibroids or endometriotic
719 tissue, peritoneal fluid), and are asked to repeatedly fill in follow-up questionnaires on their condition
720 and quality of life, so that the clinical findings can be correlated with the outcome years later.

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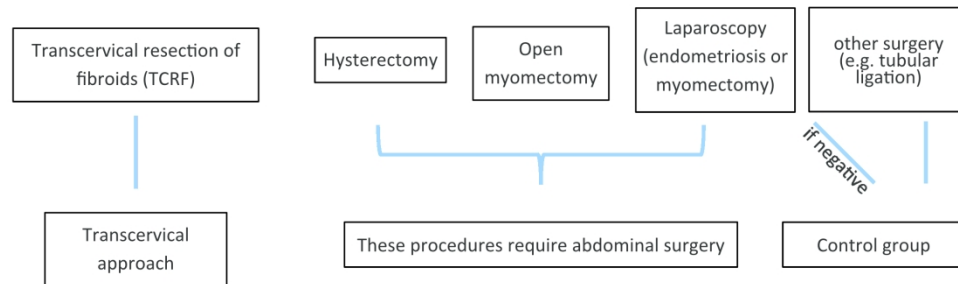


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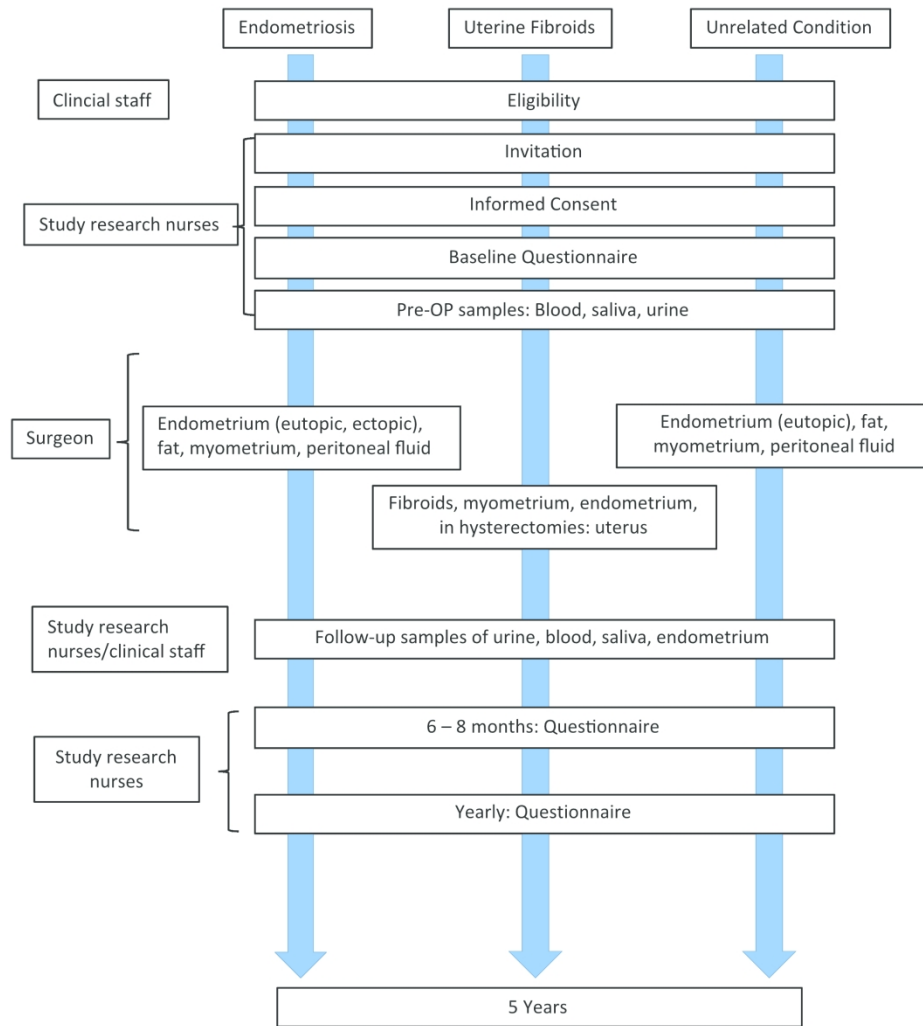


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