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Intra-operative Ultrasound Guided Laparoscopic Ovarian Cystectomy (UGLOC) as a method of fertility preservation in the management of benign ovarian cysts: a randomised controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-060409
Article Type:	Protocol
Date Submitted by the Author:	20-Dec-2021
Complete List of Authors:	Kasaven, Lorraine S; Imperial College London, Cancer and Surgery; Queen Charlotte's and Chelsea Hospital, Obstetrics and Gynaecology Jones, Benjamin; Imperial College London Ghaem-Maghami, Sadaf; Imperial College London; Imperial College Healthcare NHS Trust Verbakel, Jan; University of Oxford, Nuffield Department of Primary Care Health Sciences; KU Leuven, Department of Public Health and Primary Care El-Bahrawy, Mona; Imperial College Healthcare NHS Trust, Department of Metabolism, Digestion and Reproduction Saso, Srdjan; Imperial College Healthcare NHS Trust Yazbek, Joseph; Imperial College Healthcare NHS Trust
Keywords:	Ultrasonography < OBSTETRICS, Minimally invasive surgery < GYNAECOLOGY, Reproductive medicine < GYNAECOLOGY

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Manuscripts

BMJ Open**Intra-operative Ultrasound Guided Laparoscopic Ovarian Cystectomy (UGLOC) as a method of fertility preservation in the management of benign ovarian cysts: a randomised controlled trial**

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Word count: 3,597

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2
3 **29 Abstract**
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5 **30 Introduction:** The lifetime risk of women undergoing surgery for the presence of benign ovarian
6
7 **31** pathology in the united kingdom (UK) is 5-10%.⁽¹⁾ Despite minimally invasive surgical techniques,
8
9 **32** evidence suggests a number of healthy ovarian follicles and tissues are resected intraoperatively,
10
11 **33** resulting in subsequent decline of ovarian reserve. Increasing demand for the implementation of
12
13 **34** fertility sparing surgical techniques is therefore prevalent. This study will evaluate the effect on
14
15 **35** ovarian reserve following two different surgical interventions for the management of benign ovarian
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17 **36** cysts.

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19
20 **37 Methods and analysis:** We will conduct a two-armed randomised controlled trial comparing
21
22 **38** laparoscopic ovarian cystectomy, considered gold standard treatment as per the Royal College of
23
24 **39** Obstetricians and Gynaecologists (RCOG) Green Top guidelines for the management of benign
25
26 **40** ovarian cysts⁽¹⁾, with ultrasound guided laparoscopic ovarian cystectomy (UGLOC), a novel method
27
28 **41** of fertility sparing surgery (FSS). The primary outcome will be the difference in anti-Mullerian
29
30 **42** hormone (pmol/L) (AMH) and antral follicle count (AFC) measured 3 and 6 months post operatively
31
32 **43** from the pre-operative baseline. Secondary outcomes include assessment of various surgical and
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34 **44** histopathological outcomes including: duration of hospital stay (days), duration of surgery (mins),
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36 **45** presence of intra-operative cyst rupture (yes/no), presence of ovarian tissue within the specimen
37
38 **46** (yes/no) and the grade of follicles excised with specimen (grade 0-4). We aim to randomise 32
39
40 **47** patients over 3 years to achieve 95% power for detecting a 25% difference in the primary outcome at
41
42 **48** a significance of 5%.

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45 **49 Ethics and dissemination:** Findings will be published in peer reviewed journals and presented at
46
47 **50** national and international conferences and scientific meetings. The Chelsea NHS Research and Ethics
48
49 **51** Committee have awarded ethical approval of the study (21/LO/036).

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51 **52 Trial registration number:** NCT05032846
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57 **Key words**

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59 Intra-operative ultrasound, benign ovarian cyst, fertility preservation surgery

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For peer review only

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3 85 **Strengths and limitations of this study**
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- 5 86 • This is the only reported prospective, randomised controlled trial to assess the use of intra-
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7 87 operative ultrasound as a method of fertility preservation surgery
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9 88 • The trial will provide an evaluation of two different surgical interventions for the
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11 89 management of benign ovarian cysts, in order to optimise women's ovarian reserve
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14 90 • The intervention is non- blinded
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16 91 • Follow up of patients is limited to 6 months
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112 **Background**

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114 Within the field of reproductive medicine, advancements over the last few decades have facilitated the
115 rapidly emerging sub-specialty in gynaecology, known as fertility preservation. This includes various
116 methods to preserve reproductive tissue or gametes such as medical, surgical or laboratory techniques,
117 thus empowering women to preserve their fecundity with a view to achieving pregnancy at a later
118 date.⁽²⁾ Such techniques were initially considered for women of reproductive age diagnosed with
119 cancer, embarking on gonadotoxic treatment regimens including chemotherapy or radiotherapy, or
120 undergoing radical surgery to remove gynaecological organs, thus rendering them infertile. Thus, in
121 the context of surgical management of gynaecological cancers, there has been an increasing demand
122 for less radical procedures, with a shift towards conservative surgical methods, in order to preserve
123 the reproductive organs. In appropriately selected women, this enables the opportunity to balance the
124 risks of recurrence from disease, whilst reserving the ability to conceive in the future. As such, the
125 mainstay treatment of Borderline Ovarian Tumours for example, in women with early-stage disease,
126 non-invasive implants or for those who wish to conceive, is fertility sparing surgery (FSS). Such
127 procedures include performing a unilateral salpingo-oophorectomy (USO) or ovarian cystectomy,
128 compared to previously adopted surgical methods of radical debulking, which required bilateral
129 salpingo-oophorectomy. Evidence suggests that FSS in this context is both safe and feasible.⁽³⁾

130

131 Infertility however is no longer limited to women undergoing treatment for cancer, as evidence
132 suggests 1 in 6 women experience infertility.⁽⁴⁾ Although there are a number of causes, it is also
133 prevalent amongst women diagnosed with benign pathology, such as endometriosis or ovarian cysts.
134 Infertility can be caused either by the underlying pathology itself, or indirectly associated with the
135 surgical intervention required to treat.⁽⁵⁾ The latter is attributed to the fact that ovarian surgery, despite
136 minimally invasive techniques, results in the resection of a number of healthy ovarian follicles and
137 tissue.⁽⁶⁾ This is exemplified from a study demonstrating that Anti-Mullerian Hormone (AMH), a
138 reliable marker of ovarian reserve, is reduced post-operatively following surgery for endometriosis.⁽⁷⁾
139 Considering the lifetime risk of women undergoing surgery for the presence of benign ovarian

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3 140 pathology is 5-10%,⁽¹⁾ it is perhaps understandable why an increasing demand for the implementation
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5 141 of fertility sparing surgical techniques for women with benign pathology is also prevalent.⁽¹⁾ Such
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7 142 demand is further exacerbated by the increasing age of motherhood observed over the last few
8
9 143 decades.⁽⁸⁾ Increasing age is associated with poorer oocyte quality and yield, thereby inadvertently
10
11 144 increasing the risk of involuntary childlessness as a direct consequence of age related fertility
12
13 145 decline.⁽⁹⁾ If women delay attempting pregnancy to a later age, in addition to the risks of surgically
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15 146 induced impairment of ovarian tissue, overall chances of achieving pregnancy in the future maybe
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17 147 significantly reduced. It is imperative therefore, that fertility sparing techniques are implemented,
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19 148 where possible, in women of reproductive age in order to optimize the chances of future successful
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21 149 conception.
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26 151 **Intra-operative ultrasound**

28 152 The use of intraoperative ultrasound has been widely implemented amongst specialties.⁽¹⁰⁾ However,
29
30 153 within gynaecological surgery, it is not as commonly recognized, despite evidence that it can be used
31
32 154 as an adjunct to improving minimally invasive surgical techniques.⁽¹¹⁾ This is primarily due to the
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34 155 improved visualization of the operative field, which can assist more technically difficult surgical
35
36 156 procedures, thus minimizing intraoperative complications.⁽¹²⁾ The application of ultrasound guidance
37
38 157 within gynaecological procedures have included predominantly ovarian cyst aspiration, in vitro
39
40 158 fertilization and removal or insertion of intra uterine devices.⁽¹⁰⁾ Although pre-operative imaging
41
42 159 provides procedural planning, it cannot compare to the information gained from real time imaging.
43
44 160 For example, in previous studies, intraoperative ultrasound detected more myomas during
45
46 161 myomectomy than pre-operative transvaginal imaging.⁽¹³⁾ Furthermore, it provides the potential to
47
48 162 assess lesion margins, ensuring resection of pathology is complete with negligible damage to
49
50 163 surrounding healthy tissues.⁽¹¹⁾ This is consistent with a recent systematic review, which also
51
52 164 demonstrated that albeit a novel technique, amongst various case series, pathology can be safely
53
54 165 resected without incurring injury to healthy reproductive tissue.⁽¹¹⁾ Therefore, intraoperative
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56 166 ultrasound has the potential to improve surgical accuracy, reduce complications and improve patient
57
58 167 safety. The application of intraoperative ultrasound as an adjunct to FSS has not been widely
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3 168 researched, with only a few case series reporting surgical outcomes on patients undergoing treatment
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5 169 for pre-malignant or malignant pathology.⁽¹⁴⁾
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9 171 **Aim**

11 172 To evaluate the effect on ovarian reserve following two different surgical interventions for the
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13 management of benign ovarian cysts.
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17 175 **Primary objective**

19 176 To determine the efficacy of intra-operative ultrasound as a method of fertility preservation surgery.
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24 178 **Secondary objectives**

26 179 To determine the surgical and histopathological outcomes in women who have undergone
27
28 intraoperative ultrasound guided ovarian cystectomy and compare to the control group.
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32 182 **Hypothesis**

34 183 Intra-operative ultrasound guided laparoscopic ovarian cystectomy will preserve more healthy ovarian
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36 tissue, resulting in a significantly reduced decline of ovarian reserve observed at 3 and 6 months post
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38 operatively, as measured by AMH and antral follicle count (AFC), when compared to laparoscopic
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40 ovarian cystectomy.
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44 188 **Methods**
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49 190 **Trial Design**

51 191 This is a multi-centre randomised controlled trial with two parallel arms, comparing laparoscopic
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53 ovarian cystectomy with ultrasound guided laparoscopic ovarian cystectomy (UGLOC) for the
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55 management of benign ovarian cysts. Participants will be recruited from two tertiary gynaecology
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57 centres in the UK. Participants will be followed up at 3 and 6 months post operatively to assess
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195 markers of ovarian reserve. Recruitment will commence October 2021, with follow up and
196 assessment expected to conclude in October 2024. **Figure 1** summarises the trial design.

197

198 **Inclusion criteria**

- 199 • Females aged between 18-50 years old
- 200 • Pregnant or non- pregnant women diagnosed with a benign ovarian cyst requiring surgical
201 management
- 202 • Cyst classifications accepted: dermoid, endometrioma, teratoma, simple, serous cystadenoma
203 or mucinous cystadenoma
- 204 • Informed written consent
- 205 • Bilateral ovarian cysts

206

207 A strict criterion for the US diagnostic features will include the following:

- 208 • Cyst size $\geq 3\text{cm}$; $\leq 10\text{cm}$
- 209 • International Ovarian Tumour Analysis Benign features (IOTA B) present:
 - 210 ○ Unilocular
 - 211 ○ Solid components (largest diameter $\leq 7\text{mm}$)
 - 212 ○ Acoustic shadows
 - 213 ○ No blood flow
 - 214 ○ Smooth multilocular cyst (largest diameter $\leq 10\text{cm}$)

215

216 **Exclusion criteria**

- 217 • Cysts deemed to be clearly physiological and $< 3\text{ cm}$ in maximum diameter
- 218 • Cysts $\geq 11\text{cm}$ in maximum diameter
- 219 • Non-adnexal masses e.g. peritoneal inclusion cysts
- 220 • Cyst with features of malignancy
- 221 • The denial or withdrawal of written informed consent

222 • Women of post- menopausal or peri-menopausal status

223 • Participants unable to attend regular follow up

224

225 **Outcome measures**

226 The primary outcome of the study is assessment of ovarian reserve at 3 and 6 months post operatively.

227 This will be measured by markers of ovarian reserve including AMH (pmol/L) and AFC (n). The

228 secondary outcomes will include: length of hospital stay (days), presence of intra-operative cyst

229 rupture (yes or no), duration of surgery (minutes), presence of ovarian tissue within the specimen

230 (yes/no) and the grade of follicles excised with specimen (grade 0-4).

231

232 **Enrolment**

233 All women referred to the outpatient gynecology clinic with a suspected ovarian cyst will have a pelvic

234 transvaginal ultrasound scan (2D and 3D ultrasonography) as part of routine clinical care. If an ovarian

235 cyst is observed on ultrasound, it will be assessed according to local protocols based on simple

236 descriptors and international ovarian tumor analysis (IOTA) simple rules. Depending on the severity of

237 symptoms, nature of the cyst and whether surgical management is indicated to treat; should the woman

238 fulfil aspects of the inclusion criteria, she will be invited to participate in the study.

239

240 The study co-ordinator will be responsible for managing the registration of each participant to the trial

241 and their allocation to either treatment arm. All participants will sign a written consent form,

242 witnessed by a member of the research team, at least 24 hours after the participant information sheet

243 has been read. All consent forms will be scanned into the electronic medical notes. It will not be

244 possible to carry out any tasks pertaining to the trial, until written consent from the participant has

245 been obtained.

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247 **Randomisation**

248 A separate research team within Imperial College London Healthcare Trust, Department of Cancer

249 and Surgery will be responsible for the allocation process, by producing randomisation sealed

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3 250 envelopes in a ratio of 1:1. The team will be asked to print labels with the allocated group and fold
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5 251 them so the content cannot be seen. They will then give the folded labels back to the study co-
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7 252 ordinator, who will be asked to place them into sealed envelopes, which will be numbered
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9 253 chronologically. The co-ordinator will not be able to see the content of the labels, in order to ensure
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11 254 concealment. Given that it is necessary for the surgeon to know which operation to perform, both the
12
13 255 participant and research team will not be blinded. The allocated arm of the RCT will be recorded on
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15 256 the Trial Subject Enrolment Log. The Principal Investigator (PI) will be responsible for keeping the
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17 257 randomisation list.
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21 259 **Procedures**

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24 260 During the recruitment process, once consent to participate in the trial has been obtained, a member of
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26 261 the research team will select a sealed randomisation envelope as numbered chronologically, which will
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28 262 then assign the patient to a treatment arm. The chronological order of envelopes will prevent the member
29
30 263 of the research team performing the randomisation themselves, or from selecting another envelope,
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32 264 should they be dissatisfied with the treatment arm assigned to the participant. The participant will then
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34 265 undergo a blood test to record their baseline pre-operative AMH level. The same surgeons will operate
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36 266 on women, regardless of treatment arm assigned. This will aim to exclude bias, which may otherwise
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38 267 attribute findings to the surgeon operating. Surgery will be performed at Imperial Healthcare NHS Trusts
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40 268 and at the University College London Hospitals NHS Foundation Trust by an experienced clinician. All
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42 269 participants will attend pre-operative assessment with an anaesthetist as standard practice of care. In most
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44 270 cases, the surgical procedure is performed either as a day case or overnight stay.
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48 272 **Surgical Intervention**

49 273 *Laparoscopic ovarian cystectomy (control)*

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52 274 Participants will undergo a laparoscopic ovarian cystectomy in the absence of ultrasound guidance.
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54 275 Surgery will be performed according to basic laparoscopy principles from the British Society of
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56 276 Gynaecological Endoscopy (BSGE).
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3 278 *Ultrasound guided laparoscopic ovarian cystectomy (intervention)*
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5 279 Participants will undergo an ultrasound guided laparoscopic ovarian cystectomy. An assistant with
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7 280 competencies in pelvic ultrasound will insert a transrectal probe intraoperatively, and provide real-time
8
9 281 ultrasound images of the ovary and cyst to be resected. The continuous use of intra-operative ultrasound
10
11 282 ensures the surgeon is able to differentiate between healthy ovarian tissue and cyst content. It is often
12
13 283 necessary following peritoneal insufflation to infiltrate 500mL of normal saline (0.9%) into the pelvis
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15 284 to enhance the ultrasound image quality and transmission of the ultrasound. The surgeon will then
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17 285 perform the laparoscopic ovarian cystectomy under ultrasound guidance, following basic laparoscopy
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19 286 principles from the BSGE.
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25 288 *Follow up*
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28 289 Post operatively, patients will return to the outpatient gynaecology clinic for follow up at 3 and 6
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30 290 months, whereby the AMH level will be checked. The blood samples will be processed by Imperial
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32 291 College Healthcare Trust laboratories or University College London Hospitals NHS Foundation Trust,
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34 292 depending on the site taken, and will be discarded as per local protocol once the AMH has been
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36 293 determined. There are no specific storage or transfer requirements outside of normal practice. In
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38 294 addition, a transvaginal ultrasound scan will be performed during the follow up appointment, to
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40 295 measure AFC and assess volume of preserved ovarian tissue. Following the second follow up
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42 296 attendance at 6 months, no further input is required from the participant in the study.
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49 298 **Discontinuation or withdrawal of participants**
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51 299 Participants who give consent to their recruitment to the trial agree to the intervention, compliance
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53 300 with follow up assessments and data collection. Participants are free to withdraw at any time from the
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55 301 protocol treatment without reason. Furthermore, participants may be withdrawn from the trial by
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57 302 members of the research team, should participation in the trial no longer be deemed within the
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59 303 participant's best interest. All data captured in relation to their participation may be destroyed at their
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3 304 request. Any decision to withdraw a participant from the study will be recorded in the electronic
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5 305 clinical record (eCRF) files and medical notes. Reasons for stopping the trial include: non-
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7 306 compliance by members of the research team to adherence of the study protocol, participants
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9 307 withdrawing consent or adverse outcomes reported following intervention.
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13 309 **Patient and public involvement**

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15 310 Various participants from gynaecology outpatient clinics voluntarily attended a virtual *research focus*
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17 311 meeting, whereby their feedback regarding aspects of the study protocol were sought including:
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19 312 feasibility and acceptability of the study design, methods of monitoring ovarian reserve post
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21 313 operatively and acceptability of the intervention arm of the trial. They also participated in revising
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23 314 patient information leaflets and GP letter templates.
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27 316 **Modification of the protocol**

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29 317 Any amendments to the research protocol will be firstly agreed by the Principle Investigator (PI) and
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31 318 the study coordinators. This may include aspects of the study design, participant recruitment, sample
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33 319 size, interventions or ethics documents including participant information sheet or consent form.
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35 320 Implementation of changes made will depend on subsequent approval from the Research Ethics
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37 321 Committee. Any amendments made will be updated on the clinicaltrials.gov website accordingly.
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41 323 **Data and trial management**

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43 324 Electronic clinical record files (eCRFs) have been designed to assist data collection. Members of the
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45 325 research team will be responsible for the completion of the eCRFs, whereby the PI will ensure
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47 326 accuracy of all data reported. Members of the research team and all aspects of the study protocol will
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49 327 adhere to the principles of the General Data Protection Regulation (2016/679) and the Data Protection
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51 328 Act (2018). All personal data will be password protected and held on a database, accessible only from
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53 329 a registered NHS Trust computer. Following data collection, all information will be anonymized
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55 330 during the data analysis stage, whereby access will be restricted to the PI and study coordinator only.
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3 331 The sponsor of the study reserves the right to store all anonymized data for 10 years after the study
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5 332 has finished, in relation to data subject consent forms and ten years after the study has completed in
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7 333 relation to primary research data. Following this, the sponsor will adhere to the confidential
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9 334 information trust destruction procedures for disposal of data. A trial management group (TMG) has
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11 335 been designed including the PI, two study co-ordinators and trial staff. They are responsible for the
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13 336 day to day running of the trial. The TMG will meet every 6 weeks to discuss recruitment numbers,
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15 337 adverse events (AEs) encountered or potential amendments to the study protocol, if required. The
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17 338 study may be subject to inspection and audit by the sponsor and other regulatory bodies to ensure
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19 339 adherence to GCP and the UK Policy Framework for Health and Social Care Research for
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21 340 Health and Social Care. Direct access to source data/documents as requested will be permitted. A data
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23 341 safety monitoring board is not deemed necessary, as the study is associated with extremely low risks.
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29 343 **Safety**

30 344 Any questions concerning adverse event reporting will be directed to the PI in the first instance. For
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32 345 non-serious AEs, whether expected or not, a brief description of associated clinical symptoms with
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34 346 date and duration of onset will be documented in the medical notes. For SAEs, an SAE form will be
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36 347 completed and emailed to the PI within 24 hours. Specifically, relapse and death due to other
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38 348 pathology, and hospitalisations for elective treatment of a pre-existing condition do not need reporting
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40 349 as SAEs. All SAEs will be reported to the Chelsea Research and Ethics Committee where in the
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42 350 opinion of the PI, the event was:

- 45 351 • 'related', i.e. resulted from the administration of any of the research procedures; and,
- 47 352 • 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence.

49 353 Reports of related and unexpected SAEs will be submitted within 15 days of the PI becoming aware
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51 354 of the event, using the NRES SAE form for non-IMP studies. The PI will notify the Sponsor of all
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53 355 related and unexpected SAEs.
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359 **Sample size and power calculation**

360 The impact of laparoscopic ovarian cystectomy performed for the management of benign ovarian
361 cysts was investigated by Kwon et al,⁽¹⁵⁾ whereby AMH and AFC was measured at 3 and 6 months
362 post operatively. We considered this study most applicable for determining the power calculation of
363 our randomised controlled trial, based on the following principles: pathology of the ovarian cysts
364 were benign in nature and the sample size (n=100) is one of the largest reported in the literature
365 review.. Kwon et al deduced that AMH levels decreased on average 30.58% (+/-29.66%) between the
366 pre-operative value to the level assessed 3 months following surgery amongst 100
367 women.⁽¹⁵⁾ Specifically, the group of patients who underwent laparoscopic ovarian cystectomy for
368 benign ovarian pathology had a mean (+/-SD) serum AMH of 1.59 (+/- 1.92)(ng/ml), which is
369 equivalent to 3.57 (+/-4.31) (pmol/L).

370 At present there are no reported studies assessing the change in AMH levels following ultrasound
371 guided ovarian cystectomy. However, within our research team, we have performed a small number
372 of ultrasound-guided surgeries on 5 women, and therefore have a small data set, considered a *pilot*
373 study. Amongst the 5 women, the mean difference (+/-SD) in AMH levels measured 3 months post
374 operatively from pre-op levels was: 14.46 (+/- 21.02) pmol/L. This represents a substantial clinical
375 difference according to previous research, and would imply women being less likely to experience
376 fertility issues as they would be near the lower end of the normal range of AMH, rather than in the
377 lowest decile (ca. 0-4.5).

378 In order to calculate the sample size, we used our pilot data to derive an estimate of the mean and
379 standard deviation. We are aware from the literature that AMH is approximately log-normally
380 distributed, as demonstrated from **Figure 2**.⁽¹⁶⁾ Using this figure, we have determined the normed
381 (percentile) transformation of values from the pilot study (**Table 1**).

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385 Table 1: Estimated values of AMH differences based on normed percentile transformation

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Sample Size Calculation					
AMH Differences (pmol/L)					
Treatment Group			Control Group		
Mean	Mean (pilot)	17.06	17.06	3.57	3.57
Standard Deviation (SD)	Standard Deviation SD (pilot)	22.28	22.28	4.31	4.31
Coefficient of variation (CV) = (SD/Mean)	Standard Error (S.E)	1.31	6.18	1.08	1.21
	Lower CI		4.70	1.42	
	Upper CI		29.42	5.73	
Number of participants	Minimum (n)	5	11	11	100
	Small sample margin		2	2	

392 The minimum sample size is 11

393

394 patients. Considering the small size of the pilot and the very low risks thus far, we have included 2

395 more observations to boost power, and assume an attrition of 25%. This leads to a total of 16

396 participants per arm, with a power of 0.95 to detect a difference at the 5% level using a standard t-test

397 on the log-transformed difference in means of AMH; our robust proxy for fertility. Thus, using the

398 pilot data, we anticipate 32 participants will need to be recruited to demonstrate differential AMH

399 levels (**Table 2**). It will be necessary to perform an interim analysis, given the low number of

400 participants available for the power calculation.

401 Table 2: Power Calculation

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Attrition (assumed)	3	3
Total (n)	16	16

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Secondary Analysis

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33 415 We will exclude participants with missing data, non-compliance of follow up or adherence to the
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35 416 study protocol, or those who withdraw from the study. The primary outcome will be compared across
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37 417 treatment groups using univariate and multivariate analysis. Confidence intervals of 99% for
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39 418 dichotomous outcomes and risk ratio will be assessed. A p value of less than 0.05 will be used to
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41 419 determine statistical significance.
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43 420

Ethics and Dissemination

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46
47 422 This study will be conducted in accordance with the principles of Good Clinical Practice. This
48
49 423 protocol was submitted to the Health Research Authority and Chelsea Research Ethics Committee,
50
51 424 whereby a favourable ethical opinion was granted. The reference number is 21/LO/036. Subsequent
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53 425 approval by individual ethical committee and competent authority was granted. Any modification to
54
55 426 the protocol will be updated on the ClinicalTrials.gov website and disseminated to all relevant parties
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3 427 (NCT05032846). The results will be published in peer-reviewed journals and disseminated at national
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5 428 and international conferences.

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9 430 **Data sharing**

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11 431 For the purpose of this publication, data sharing is not applicable as no datasets have been generated
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13 432 and/or analysed as yet.

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17 434 **Discussion**

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19 435 Fertility sparing surgery has evolved immensely since it was first introduced for the management of
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21 436 gynaecological cancers in women of reproductive age. As growing evidence suggests it is a safe and
22
23 437 feasible option in women of reproductive age diagnosed with Borderline Ovarian Tumours or early
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25 438 stage ovarian carcinoma, few studies assess the use of fertility sparing surgery for the management of
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27 439 benign pathology. However, considering the causes of infertility include a number of benign
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29 440 pathologies, alongside the increasing risk of age related fertility decline associated with delayed child
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31 441 bearing, the demand to implement fertility sparing techniques is imminently growing amongst women
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33 442 undergoing surgery for benign pathology during their reproductive years.

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37 444 Recent advancements within ultrasound technology have facilitated the enhancement in diagnostic
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39 445 accuracy, as evidenced by the detection of smaller ovarian lesions or pathology on ultrasound scan.⁽¹⁴⁾
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41 446 Furthermore, the ability to delineate cyst content from healthy ovarian tissue also reduces the risk of
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43 447 cyst rupture; which depending on the content may have detrimental effects on the pelvis. Considering
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45 448 evidence suggests a significantly higher volume of normal ovarian tissue is resected during
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47 449 cystectomy of endometriomas when evaluated histologically,⁽¹⁷⁾ the ability to preserve optimal healthy
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49 450 ovarian tissue is also a benefit of intra-operative ultrasound. Therefore, combining this diagnostic tool
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51 451 with FSS, provides an apt alternative to the otherwise *blind* resection of healthy ovarian tissue during
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53 452 ovarian cystectomy.

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3 454 The PI and co-investigators of this study have previously published the outcomes of ultrasound guided
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5 455 laparoscopic ovarian wedge resection for the management of recurrent serous borderline ovarian
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7 456 tumours⁽¹⁴⁾ and in the context of treatment for anti-NMDA receptor encephalitis.⁽¹⁸⁾ Whilst both surgical
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9 457 and oncological outcomes reported were successful, there was no measurable effect on the ovarian
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11 458 reserve assessed post-operatively. This prospective trial therefore, will evaluate the efficacy of this
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13 459 method of FSS and provide real-time evidence for the post-operative effects on ovarian reserve. In
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15 460 addition, findings from the study will be able to deduce whether the type of ovarian cyst resected is
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17 461 associated with loss of ovarian reserve, allowing clinicians to provide informative counselling, so that
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19 462 women can make well informed decisions regarding their future fertility before deciding to undergo
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21 463 surgery. Furthermore, the technique is readily available and considered a low cost treatment option for
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23 464 the management of benign ovarian cysts. Particularly, considering transvaginal ultrasound scanning is
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25 465 a competency acquired by all UK trainees, and therefore many specialists already attain the skills to
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27 466 implement this method of FSS. The procedure is therefore considered to be widely applicable
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29 467 nationally.
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34 469 **Author's contributions**

35
36 470 JY and LSK conceived and designed the study. LSK drafted the trial protocol. JY, JV and MEB
37
38 471 provided methodological and statistical expertise. JY and SGM provide expertise in clinical outcomes
39
40 472 following ultrasound. LSK and BPJ drafted the manuscript. LSK and SS with the support of the trial
41
42 473 Principal Investigator, have responsibilities for day-to-day running of the trial including participant
43
44 474 recruitment, data collection and liaising with other sites. All authors critically reviewed and approved
45
46 475 the final version of the manuscript.
47
48
49

50 476 51 477 **Funding**

52
53 478 This research received no specific grant from any funding agency in the public, commercial or not-
54
55 479 for-profit sectors
56
57

58 480 59 481 **Disclaimer**

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482 The views expressed in this publication are those of the author (s)

483

484 **Competing interests**

485 None declared.

486

487 **Provenance and peer review**

488 Not commissioned; externally peer reviewed.

489

490 **Trial Personnel**

491 See **Table 3.**

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For peer review only

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Table 3: Trial Personnel

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Histopathologist	Professor Mona El-Bahrawy Professor of Pathology Department of Metabolism, Digestion and Reproduction, Imperial College London m.elbahrawy@imperial.ac.uk

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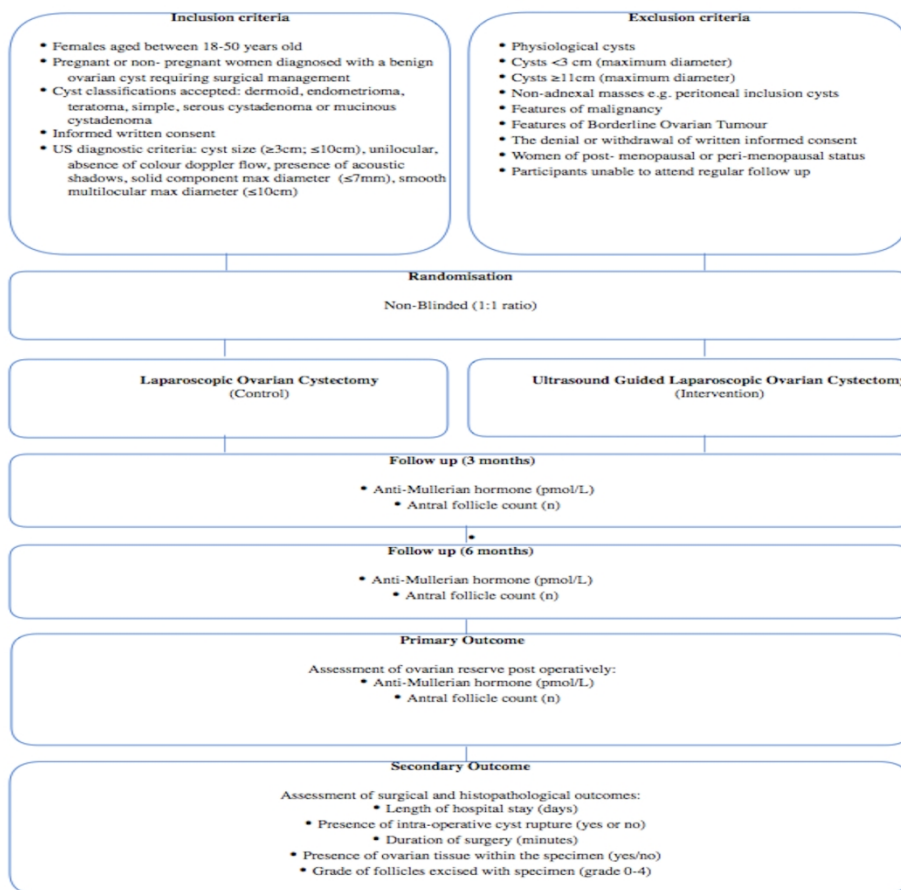
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519 **References**

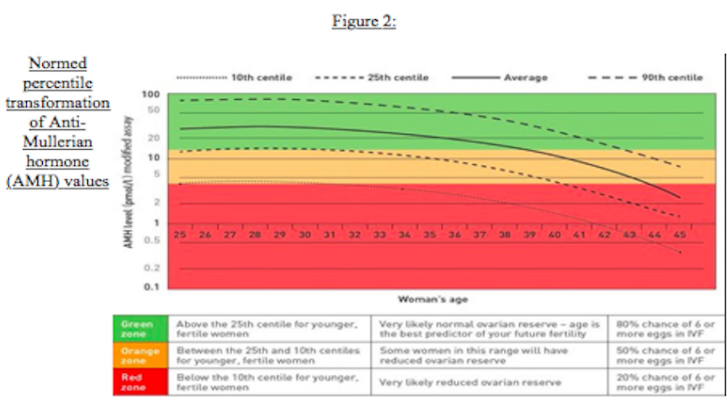
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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	8
	4b	Settings and locations where the data were collected	10
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	12
Sample size	7a	How sample size was determined	13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	14
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	10
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	10
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	10

1		assessing outcomes) and how	
2	11b	If relevant, description of the similarity of interventions	10
3	Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes
4		12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses
5			15
6	Results		
7	Participant flow (a	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and
8	diagram is strongly		were analysed for the primary outcome
9	recommended)	13b	For each group, losses and exclusions after randomisation, together with reasons
10			NA
11	Recruitment	14a	Dates defining the periods of recruitment and follow-up
12		14b	Why the trial ended or was stopped
13			8
14	Baseline data	15	A table showing baseline demographic and clinical characteristics for each group
15	Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was
16			by original assigned groups
17	Outcomes and	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its
18	estimation		precision (such as 95% confidence interval)
19		17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended
20			NA
21	Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing
22			pre-specified from exploratory
23			NA
24	Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)
25			NA
26	Discussion		
27	Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses
28	Generalisability	21	Generalisability (external validity, applicability) of the trial findings
29	Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence
30			NA
31	Other information		
32	Registration	23	Registration number and name of trial registry
33			15
34	Protocol	24	Where the full trial protocol can be accessed, if available
35	Funding	25	Sources of funding and other support (such as supply of drugs), role of funders
36			17

37 *We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also
 38 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.
 39 Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.
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BMJ Open

Study Protocol for a Randomised Controlled Trial on the use of Intra-operative Ultrasound Guided Laparoscopic Ovarian Cystectomy (UGLOC) as a method of fertility preservation in the management of benign ovarian cysts

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-060409.R1
Article Type:	Protocol
Date Submitted by the Author:	04-Jun-2022
Complete List of Authors:	Kasaven, Lorraine S; Imperial College London, Cancer and Surgery; Queen Charlotte's and Chelsea Hospital, Obstetrics and Gynaecology Jones, Benjamin; Imperial College London Ghaem-Maghami, Sadaf; Imperial College London; Imperial College Healthcare NHS Trust Verbakel, Jan; University of Oxford, Nuffield Department of Primary Care Health Sciences; KU Leuven, Department of Public Health and Primary Care El-Bahrawy, Mona; Imperial College Healthcare NHS Trust, Department of Metabolism, Digestion and Reproduction Saso, Srdjan; Imperial College Healthcare NHS Trust Yazbek, Joseph; Imperial College Healthcare NHS Trust
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Obstetrics and gynaecology, Research methods, Surgery
Keywords:	Ultrasonography < OBSTETRICS, Minimally invasive surgery < GYNAECOLOGY, Reproductive medicine < GYNAECOLOGY

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Manuscripts

BMJ Open**Study Protocol for a Randomised Controlled Trial on the use of Intra-operative Ultrasound Guided Laparoscopic Ovarian Cystectomy (UGLOC) as a method of fertility preservation in the management of benign ovarian cysts**

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Word count: 3,597

29 Abstract

30 **Introduction:** The lifetime risk of women undergoing surgery for the presence of benign ovarian
31 pathology in the united kingdom (UK) is 5-10%. Despite minimally invasive surgical techniques,
32 evidence suggests a number of healthy ovarian follicles and tissues are resected intraoperatively,
33 resulting in subsequent decline of ovarian reserve. As such, there is an increasing demand for the
34 implementation of fertility preservation surgery (FPS). This study will evaluate the effect on ovarian
35 reserve following two different surgical interventions for the management of benign ovarian cysts.

36 **Methods and analysis:** We will conduct a two-armed randomised controlled trial comparing
37 laparoscopic ovarian cystectomy, considered gold standard treatment as per the Royal College of
38 Obstetricians and Gynaecologists (RCOG) Green Top guidelines for the management of benign
39 ovarian cysts, with ultrasound guided laparoscopic ovarian cystectomy (UGLOC), a novel method of
40 FPS. The study commencement date was October 2021 and completion date October 2024. The
41 primary outcome will be the difference in anti-Mullerian hormone (pmol/L) (AMH) and antral follicle
42 count (AFC) measured 3 and 6 months post operatively from the pre-operative baseline. Secondary
43 outcomes include assessment of various surgical and histopathological outcomes including: duration
44 of hospital stay (days), duration of surgery (mins), presence of intra-operative cyst rupture (yes/no),
45 presence of ovarian tissue within the specimen (yes/no) and the grade of follicles excised with
46 specimen (grade 0-4). We aim to randomise 94 patients over 3 years to achieve power of 80% at an
47 alpha level of 0.05.

48 **Ethics and dissemination:** Findings will be published in peer reviewed journals and presented at
49 national and international conferences and scientific meetings. The Chelsea NHS Research and Ethics
50 Committee have awarded ethical approval of the study (21/LO/036).

51 **Trial registration number:** NCT05032846

53 Key words

55 Intra-operative ultrasound, benign ovarian cyst, fertility preservation surgery

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3 57 **Strengths and limitations of this study**
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- 5 58 • This is the only reported prospective, randomised controlled trial to assess the use of intra-
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7 59 operative ultrasound as a method of fertility preservation surgery
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9 60 • The trial will provide an evaluation of two different surgical interventions for the
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11 61 management of benign ovarian cysts, in order to optimise women's ovarian reserve
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14 62 • The intervention is non-blinded
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16 63 • Follow up of women is limited to 6 months
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84 **Background**

85 Within the field of reproductive medicine, advancements over the last few decades have facilitated the
86 rapidly emerging area of expertise, referred to as fertility preservation. This includes various methods
87 to preserve reproductive tissue or gametes such as medical, surgical or laboratory techniques, thus
88 empowering women to preserve their fecundity with a view to achieving pregnancy at a later date.⁽¹⁾
89 Such techniques were initially considered for women of reproductive age diagnosed with cancer,
90 embarking on gonadotoxic treatment regimens including chemotherapy or radiotherapy, or
91 undergoing radical surgery to remove gynaecological organs, thus rendering them infertile. Thus, in
92 the context of surgical management of gynaecological cancers, there has been an increasing demand
93 for less radical procedures, with a shift towards conservative surgical methods, in order to preserve
94 the reproductive organs. In appropriately selected women, this enables the opportunity to balance the
95 risks of recurrence from disease, whilst reserving the ability to conceive in the future. As such, the
96 mainstay treatment of Borderline Ovarian Tumours for example, in women with early-stage disease,
97 non-invasive implants or for those who wish to conceive, is fertility preservation surgery (FPS). Such
98 procedures include performing a unilateral salpingo-oophorectomy (USO) or ovarian cystectomy,
99 compared to previously adopted surgical methods of radical debulking, which required bilateral
100 salpingo-oophorectomy. Evidence suggests that in this context, FPS is both safe and feasible.⁽²⁾
101
102 Consideration of fertility however, is no longer limited to women undergoing treatment for cancer, as
103 evidence suggests 1 in 6 women now experience infertility.⁽³⁾ Although there are a number of causes,
104 it is also prevalent amongst women diagnosed with benign pathology, such as endometriosis or
105 ovarian cysts. Infertility can be caused either by the underlying pathology itself, or indirectly
106 associated with the surgical intervention required to treat.⁽⁴⁾ The latter is attributed to the fact that
107 ovarian surgery, despite minimally invasive techniques, results in the resection of a number of healthy
108 ovarian follicles and tissue.⁽⁵⁾ This is exemplified from a study demonstrating that anti-Mullerian
109 hormone (AMH), a reliable marker of ovarian reserve, is reduced post-operatively following surgery
110 for endometriosis.⁽⁶⁾ Considering the lifetime risk of women undergoing surgery for the presence of
111 benign ovarian pathology is 5-10%,⁽⁷⁾ it is perhaps understandable why there is an increasing demand

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3 112 for fertility preserving surgical techniques for women with benign pathology.⁽⁸⁾ Such demand is
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5 113 further exacerbated by the increasing age of motherhood observed over the last few decades.⁽⁹⁾
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7 114 Increasing age is associated with poorer oocyte quality and yield, thereby inadvertently increasing the
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9 115 risk of involuntary childlessness as a direct consequence of age related fertility decline.⁽¹⁰⁾ If women
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11 116 delay attempting pregnancy to a later age, in addition to the risks of surgically induced impairment of
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13 117 ovarian tissue, overall chances of achieving pregnancy in the future maybe significantly reduced. It is
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15 118 imperative therefore, that fertility preserving techniques are implemented, where possible, in women
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17 119 of reproductive age in order to optimize the chances of future successful conception.
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121 **Intra-operative ultrasound**

122 The use of intraoperative ultrasound has been widely implemented, with frequent use observed in the
123 resection of hepatic metastatic disease, neuroendocrine tumours from the pancreas and renal cell
124 carcinoma.^(11, 12) However, within gynaecological surgery, it is not as commonly recognized, despite
125 evidence that it can be used as an adjunct to improving minimally invasive surgical techniques.⁽¹³⁾
126 This is primarily due to the improved visualization of the operative field, which can assist more
127 technically difficult surgical procedures, thus minimizing intraoperative complications and injury to
128 surrounding vessels and organs.⁽¹⁴⁾ The application of ultrasound guidance within gynaecological
129 procedures have included predominantly ovarian cyst aspiration, in vitro fertilization and removal or
130 insertion of intra uterine devices and in fertility preservation surgery for Borderline Ovarian
131 Tumours.^(11, 15) Although pre-operative imaging provides procedural planning, it cannot compare to
132 the information gained from real time imaging. For example, in previous studies, intraoperative
133 ultrasound detected more myomas during myomectomy than pre-operative transvaginal imaging.⁽¹⁶⁾
134 Furthermore, it provides the potential to assess lesion margins, ensuring resection of pathology is
135 complete with negligible damage to surrounding healthy tissues.⁽¹³⁾ This is consistent with a recent
136 systematic review, which also demonstrated that albeit a novel technique, amongst various case series,
137 pathology can be safely resected without incurring injury to healthy reproductive tissue as
138 differentiation between pathology and healthy ovarian tissue could clearly be defined on scan.⁽¹³⁾ The
139 application of intraoperative ultrasound as an adjunct to FPS has not been widely researched, with

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3 140 only a few case series reporting surgical outcomes on patients undergoing treatment for pre-malignant
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5 141 or malignant pathology.⁽¹⁷⁾
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9 143 **Aim**

11 144 To compare the effect of two different surgical interventions, including either laparoscopic ovarian
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13 145 cystectomy (control group) or ultrasound guided laparoscopic ovarian cystectomy (UGLOC)
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15 146 (experimental group) for the management of benign ovarian cysts, on the ovarian reserve measured 3
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17 147 and 6 months post operatively.
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21 149 **Primary objective**

23 150 To compare the difference in serum AMH level and AFC number at 3 and 6 months post operatively
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25 151 in women who have undergone UGLOC and compare to the control group.
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30 154 **Secondary objectives**

32 155 Secondary outcomes include assessment of various surgical and histopathological outcomes
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34 156 including: duration of hospital stay (days), duration of surgery (mins), presence of intra-operative cyst
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36 157 rupture (yes/no), presence of ovarian tissue within the specimen (yes/no) and the grade of follicles
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38 158 excised with specimen (grade 0-4).
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43 161 **Hypothesis**

45 162 The difference in serum AMH level and AFC number measured at 3 and 6 months post operatively
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47 163 will be significantly less following UGLOC when compared to laparoscopic ovarian cystectomy.
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51 166 **Methods**

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53 168 **Trial Design**

54 169 This is a single-centre randomised controlled trial with two parallel arms, comparing laparoscopic
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56 170 ovarian cystectomy with UGLOC for the management of benign ovarian cysts. Women will be
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3 168 recruited from one tertiary gynaecology centre in the UK. They will be followed up at 3 and 6 months
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5 169 post operatively to assess markers of ovarian reserve. Recruitment will commence from October
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7 170 2021, with follow up and assessment expected to conclude in October 2024. **Figure 1** summarises the
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9 171 trial design.

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12 17213
14 173 **Inclusion criteria**

- 15 174 • Women aged between 18-45 years old
- 16 175 • Non- pregnant women diagnosed with a benign ovarian cyst requiring surgical management
- 17 176 • Cyst classifications accepted: mature teratoma (dermoid), simple cyst, serous cystadenoma or
18 177 mucinous cystadenoma
- 19 178 • Informed written consent

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22 17923
24 180 A strict criterion for the US diagnostic features will include the following:

- 25 181 • Cyst size ≥ 3 cm; ≤ 10 cm
- 26 182 • International Ovarian Tumour Analysis Benign features (IOTA B) present:
 - 27 183 ○ Unilocular
 - 28 184 ○ Solid components (largest diameter ≤ 7 mm)
 - 29 185 ○ Acoustic shadows
 - 30 186 ○ No blood flow
 - 31 187 ○ Smooth multilocular cyst (largest diameter ≤ 10 cm)

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34 18835
36 189 **Exclusion criteria**

- 37 190 • Cysts deemed to be clearly physiological and < 3 cm in maximum diameter
- 38 191 • Cysts ≥ 11 cm in maximum diameter
- 39 192 • Bilateral ovarian cysts
- 40 193 • Non-adnexal masses e.g. peritoneal inclusion cysts
- 41 194 • Cyst with features of malignancy

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- 195 • Endometrioma or fimbrial cysts
- 196 • The denial or withdrawal of written informed consent
- 197 • Pregnant women
- 198 • Women of post- menopausal or peri-menopausal status
- 199 • Women unable to attend regular follow up

200

201 **Outcome measures**

202 The primary outcome of the study is the assessment of ovarian reserve at 3 and 6 months post
203 operatively. This will be assessed by measuring the AMH (pmol/L) level and AFC (n). The secondary
204 outcomes will include: length of hospital stay (days), presence of intra-operative cyst rupture (yes or
205 no), duration of surgery (minutes), presence of ovarian tissue within the specimen (yes/no) and the
206 grade of follicles excised with the specimen (grade 0-4).

207

208 **Enrolment**

209 All women referred to the outpatient gynecology clinic with a suspected ovarian cyst will have a
210 pelvic transvaginal ultrasound scan (2D and 3D ultrasonography) as part of routine clinical care. If an
211 ovarian cyst is diagnosed on ultrasound, it will be assessed according to local protocols based on
212 simple descriptors and international ovarian tumor analysis (IOTA) simple rules. Depending on the
213 severity of symptoms, nature of the cyst and whether surgical management is indicated to treat;
214 should the woman fulfil aspects of the inclusion criteria, she will be invited to participate in the study.
215 Any woman at the upper age limit for inclusion in the study who also presents with a history of
216 climacteric symptoms, irregular periods or has an AFC ≤ 4 ⁽¹⁸⁾ should be considered of peri
217 menopausal or menopausal status, and thus not eligible for recruitment to the study.

218

219 The study co-ordinator will be responsible for managing the registration of each participant to the trial
220 and their allocation to either treatment arm. All participants will sign a written consent form,
221 witnessed by a member of the research team, at least 24 hours after the participant information sheet

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3 222 has been read. All consent forms will be scanned into the electronic medical notes. It will not be
4
5 223 possible to carry out any tasks pertaining to the trial, until written consent from the participant has
6
7 224 been obtained.
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9 225

11 226 **Randomisation**

13 227 A separate research team within Imperial College London Healthcare Trust, Department of Cancer
14
15 228 and Surgery will be responsible for the allocation process, by producing randomisation sealed
16
17 229 envelopes in a ratio of 1:1. The team will be asked to print labels with the allocated group and fold
18
19 230 them so the content cannot be seen. They will then give the folded labels back to the study co-
20
21 231 ordinator, who will be asked to place them into opaque sealed envelopes, which will be numbered in
22
23 232 ascending order. The co-ordinator will not be able to see the content of the labels, in order to ensure
24
25 233 concealment. Given that it is necessary for the surgeon to know which operation to perform, both the
26
27 234 participant and research team will not be blinded. The allocated arm of the RCT will be recorded on
28
29 235 the Trial Subject Enrolment Log. The Principal Investigator (PI) will be responsible for keeping the
30
31 236 randomisation list.
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37 238 **Procedures**

39 239 During the recruitment process, once consent to participate in the trial has been obtained, a member of
40
41 240 the research team will select a sealed randomisation envelope as numbered in ascending order, which
42
43 241 will then assign the participant to a treatment arm. The ascending order of envelopes will prevent the
44
45 242 member of the research team performing the randomisation themselves, or from selecting another
46
47 243 envelope, should they be dissatisfied with the treatment arm assigned to the participant. The participant
48
49 244 will then undergo a blood test to record their baseline pre-operative AMH level. The same surgeons will
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51 245 operate on all participants, regardless of treatment arm assigned. This will aim to exclude bias, which
52
53 246 may otherwise attribute findings to the surgeon operating. Surgery will be performed at Imperial
54
55 247 Healthcare NHS Trusts by an experienced clinician. All participants will attend pre-operative assessment
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57 248 with an anaesthetist as standard practice of care. All women undergoing surgery will be required to have
58
59 249 an overnight stay in hospital.
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45 251 **Surgical Intervention**6
7 252 *Laparoscopic ovarian cystectomy (control)*

8
9 253 Laparoscopic entry will be performed according to basic laparoscopic principles from the British Society
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11 254 of Gynaecological Endoscopy (BSGE).⁽⁷⁾ Pneumoperitoneum is achieved through infiltration of carbon
12
13 255 dioxide into the pelvis, which provides insufflation and visualization of the pelvic organs. Participants
14
15 256 will undergo laparoscopic ovarian cystectomy in the absence of ultrasound guidance. Following removal
16
17 257 of the cyst through laparoscopic specimen retrieval bags, routine closure is performed.

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22 259 *Ultrasound guided laparoscopic ovarian cystectomy (intervention)*

23
24 260 Laparoscopic entry and peritoneal insufflation of carbon dioxide are performed to achieve
25
26 261 pneumoperitoneum. Following laparoscopic entry and assessment of the operating field, 500mLs of
27
28 262 normal saline (0.9%) is infiltrated into the pelvis, for enhancement of the ultrasound image quality and
29
30 263 transmission of the ultrasound. This remains within the pelvis during the course of the operation. An
31
32 264 assistant with competencies in pelvic ultrasound scanning will insert a transrectal probe intraoperatively,
33
34 265 and provide real-time ultrasound images of the ovary and cyst to be resected. A non-traumatic instrument
35
36 266 is used to locate the cyst, whilst correlating between the laparoscopic and ultrasound images. The
37
38 267 cystectomy is performed under continuous ultrasound guidance, above the level of the saline solution,
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40 268 ensuring the surgeon is able to differentiate between healthy ovarian tissue and cyst content. Following
41
42 269 removal of the cyst through laparoscopic specimen retrieval bags, routine closure is performed.

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47 271 *Follow up*

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49
50 272 Post operatively, participants will return to the outpatient gynaecology clinic for follow up at 3 and 6
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52 273 months, whereby the AMH level will be assessed. The blood samples will be processed by Imperial
53
54 274 College Healthcare Trust laboratories and will be discarded as per local protocol once the AMH has
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56 275 been determined. There are no specific storage or transfer requirements outside of normal practice. In
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58 276 addition, a transvaginal ultrasound scan will be performed during the follow up appointment, to
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3 277 measure AFC and assess volume of preserved ovarian tissue. Following the second follow up
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5 278 attendance at 6 months, no further input is required from the participant in the study.
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10 11 280 **Discontinuation or withdrawal of participants**

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13 281 Participants who give consent to their recruitment to the trial agree to the intervention, compliance
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15 282 with follow up assessments and data collection. Participants are free to withdraw at any time from the
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17 283 protocol treatment without reason. Furthermore, participants may be withdrawn from the trial by
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19 284 members of the research team, should participation in the trial no longer be deemed within the
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21 285 participant's best interest. All data captured in relation to their participation may be destroyed at their
22
23 286 request. Any decision to withdraw a participant from the study will be recorded in the electronic
24
25 287 clinical record (eCRF) files and medical notes. Reasons for stopping the trial include: non-
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27 288 compliance by members of the research team to adherence of the study protocol, participants
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29 289 withdrawing consent or adverse outcomes reported following intervention.
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34 291 **Patient and public involvement**

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36 292 Ten women were approached by members of the research team from outpatient Gynaecology clinics,
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38 293 to request their assistance reviewing participant information resources applicable to the study. If
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40 294 agreeable, they were provided with a copy of the patient information leaflet and consent form to
41
42 295 review over the course of a week. They then voluntarily attended a virtual *research focus* meeting,
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44 296 whereby their feedback regarding aspects of the study protocol were sought including: feasibility and
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46 297 acceptability of the study design, methods of monitoring ovarian reserve post operatively and
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48 298 acceptability of the intervention arm of the trial. They also participated in revising patient information
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50 299 leaflets and GP letter templates.
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54 301 **Modification of the protocol**

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56 302 Any amendments to the research protocol will be firstly agreed by the Principle Investigator (PI) and
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58 303 the study coordinators. This may include aspects of the study design, participant recruitment, sample
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3 304 size, interventions or ethics documents including participant information sheet or consent form.
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5 305 Implementation of changes made will depend on subsequent approval from the Research Ethics
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7 306 Committee. Any amendments made will be updated on the clinicaltrials.gov website accordingly.
8

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11 308 **Data and trial management**

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13 309 Electronic clinical record files (eCRFs) have been designed to assist data collection. Members of the
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15 310 research team will be responsible for the completion of the eCRFs, whereby the PI will ensure
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17 311 accuracy of all data reported. Members of the research team and all aspects of the study protocol will
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19 312 adhere to the principles of the General Data Protection Regulation (2016/679) and the Data Protection
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21 313 Act (2018). All personal data will be password protected and held on a database, accessible only from
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23 314 a registered NHS Trust computer. Following data collection, all information will be anonymized
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25 315 during the data analysis stage, whereby access will be restricted to the PI and study coordinator only.
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27 316 The sponsor of the study reserves the right to store all anonymized data for 10 years after the study
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29 317 has finished, in relation to data subject consent forms and ten years after the study has completed in
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31 318 relation to primary research data. Following this, the sponsor will adhere to the confidential
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33 319 information trust destruction procedures for disposal of data. A trial management group (TMG) has
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35 320 been designed including the PI, two study co-ordinators and trial staff. They are responsible for the
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37 321 day to day running of the trial. The TMG will meet every 6 weeks to discuss recruitment numbers,
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39 322 adverse events (AEs) encountered or potential amendments to the study protocol, if required. The
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41 323 study may be subject to inspection and audit by the sponsor and other regulatory bodies to ensure
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43 324 adherence to GCP and the UK Policy Framework for Health and Social Care Research for
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45 325 Health and Social Care. Direct access to source data/documents as requested will be permitted. A data
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47 326 safety monitoring board is not deemed necessary, as the study is associated with extremely low risks.
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52 328 **Safety**

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54 329 Any questions concerning adverse event reporting will be directed to the PI in the first instance. For
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56 330 non-serious AEs, whether expected or not, a brief description of associated clinical symptoms with
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58 331 date and duration of onset will be documented in the medical notes. For SAEs, an SAE form will be
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3 332 completed and emailed to the PI within 24 hours. Specifically, relapse and death due to other
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5 333 pathology, and hospitalisations for elective treatment of a pre-existing condition do not need reporting
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7 334 as SAEs. All SAEs will be reported to the Chelsea Research and Ethics Committee where in the
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9 335 opinion of the PI, the event was:

AMH Differences (pmol/L)

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- ‘related’, i.e. resulted from the administration of any of the research procedures; and,
 - ‘unexpected’, i.e. an event that is not listed in the protocol as an expected occurrence.
- 14
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17 338 Reports of related and unexpected SAEs will be submitted within 15 days of the PI becoming aware
18
19 339 of the event, using the NRES SAE form for non-IMP studies. The PI will notify the Sponsor of all
20
21 340 related and unexpected SAEs.
22

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26 342 **Sample size and power calculation**

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28 343 The impact of laparoscopic ovarian cystectomy performed for the management of benign ovarian
29
30 344 cysts has been investigated by Kwon et al,⁽¹⁹⁾ whereby AMH and AFC was measured at 3 and 6
31
32 345 months post operatively. We considered this study most applicable for determining the power
33
34 346 calculation of our randomised controlled trial, based on the following principles: pathology of the
35
36 347 ovarian cysts were benign in nature and the sample size (n=100) is one of the largest reported. Kwon
37
38 348 et al deduced that AMH levels decreased on average 30.58% (+/-29.66%) between the pre-operative
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40 349 value to the level assessed 3 months following surgery.⁽¹⁹⁾ Specifically, the group of women who
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42 350 underwent laparoscopic ovarian cystectomy for benign ovarian pathology had a mean (+/-SD) serum
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44 351 AMH of 1.59 (+/- 1.92)(ng/ml), equivalent to 3.57 (+/-4.31) (pmol/L).

45
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48 352 At present there are no reported studies assessing the difference in AMH levels following ultrasound
49
50 353 guided ovarian cystectomy. We have performed a small *pilot* study consisting of 5 women who have
51
52 354 undergone ultrasound-guided ovarian cystectomy for borderline ovarian tumours. In order to calculate
53
54 355 the sample size, we used our pilot data to derive an estimate of the mean and standard deviation.

55
56 356 **(Table 1)**
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60

	Treatment Group	Control Group
	(Pilot)	(Kwon et al)
Mean	17.06	3.57
Standard Deviation (SD)	22.28	4.31
Coefficient of variation (CV) = (SD/Mean)	1.31	1.21
Number of participants	5	100

Table 1: Pilot data compared to control study

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365 A power calculation was performed using the TrialSize package for a 2 sample mean for superiority
 366 or non-inferiority trials with R Statistical Programming (version 4.2.0). We assumed a power of 80%
 367 at an alpha level of 0.05 (two tailed), based on a superiority margin of 2.0 pmol/L in AMH,
 368 considered to be a significant difference between the two treatment groups in the primary outcome.

369

370 Referring to Table 1, the true mean AMH difference between the groups is 13.49 pmol/L, and the
 371 pooled standard deviation of the two groups 22.44 pmol/L. Thus, the total number of participants

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3 372 required for each treatment arm is 47, or 94 in total. This is based on a 1:1 randomisation of
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5 373 participants, which should also factor for variation in baseline characteristics. To account for a 5%
6
7 374 drop out and 10% loss to follow-up rate, we will recruit 108 participants into the study, or 54 per
8
9 375 group.

10 376

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12
13 377 It will be necessary to perform an interim analysis to re-assess the standard deviation, given the low
14
15 378 number of participants available for the power calculation.

19 379 **Secondary Analysis**

20
21 380 We will exclude participants with missing data, non-compliance of follow up or adherence to the
22
23 381 study protocol, or those who withdraw from the study. The primary outcome will be compared across
24
25 382 treatment groups using univariate and multivariate analysis. Confidence intervals of 99% for
26
27 383 dichotomous outcomes and risk ratio will be assessed. A p value of less than 0.05 will be used to
28
29 384 determine statistical significance.

30 385

34 386 **Ethics and Dissemination**

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36 387 This study will be conducted in accordance with the principles of Good Clinical Practice. This
37
38 388 protocol was submitted to the Health Research Authority and Chelsea Research Ethics Committee,
39
40 389 whereby a favourable ethical opinion was granted. The reference number is 21/LO/036. Subsequent
41
42 390 approval by an individual ethical committee and competent authority was granted. Any modification
43
44 391 to the protocol will be updated on the ClinicalTrials.gov website and disseminated to all relevant
45
46 392 parties (NCT05032846). The results will be published in peer-reviewed journals and disseminated at
47
48 393 national and international conferences.

49 394

52 395 **Data sharing**

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54 396 For the purpose of this publication, data sharing is not applicable as no datasets have been generated
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56 397 and/or analysed as yet.

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399 **Potential biases within the study**

400 Evidence suggests the type of ovarian cyst resected determines the magnitude of decline in ovarian
401 reserve. For example, surgical resection of endometriomas, are associated with the greatest degree of
402 decline in AMH, compared to cysts of other pathology.⁽²⁰⁾ This is most likely because the underlying
403 pathogenesis of endometriosis itself, causes adhesions complicating the procedure and increases the
404 volume of healthy ovarian stroma resected within the cyst contents.^(19, 21) For this reason we have
405 excluded endometriomas from the study.

406
407 Furthermore, certain participant characteristics may lead to bias in the study, such as the ethnicity and
408 age of the participant recruited. These are potential confounding variables, particularly as AMH levels
409 are influenced by both age related and racial disparities.⁽²²⁾ Therefore it is appropriate during data
410 analysis, to perform a separate subgroup analysis to determine whether certain demographics are
411 associated with degree of decline of AMH between the control and experimental groups.

413 **Discussion**

414
415 Fertility preservation surgery has evolved immensely since it was first introduced for the management
416 of gynaecological cancers in women of reproductive age. As evidence continues to propagate, it is
417 considered a safe and feasible option in women of reproductive age diagnosed with Borderline
418 Ovarian Tumours or early stage ovarian carcinoma. Few studies however, assess the use of FPS for
419 the management of benign ovarian pathology. Considering the causes of infertility include a number
420 of benign pathologies, alongside the increasing risk of age related fertility decline associated with
421 delayed child bearing, the demand for fertility preserving techniques is imminently growing amongst
422 women undergoing surgery for benign pathology during their reproductive years.

423
424 Recent advancements within ultrasound technology have facilitated the enhancement in diagnostic
425 accuracy, as evidenced by the detection of smaller ovarian lesions or pathology on ultrasound scan.⁽¹⁷⁾

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3 426 Furthermore, the ability to delineate cyst content from healthy ovarian tissue also reduces the risk of
4
5 427 cyst rupture; which depending on the contents may have detrimental effects on the pelvis. The ability
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7 428 to preserve optimal healthy ovarian tissue is therefore a benefit of intra-operative ultrasound,
8
9 429 providing an apt alternative to the otherwise *blind* resection of healthy ovarian tissue during ovarian
10
11 430 cystectomy.

12 431
13
14 432 The PI and co-investigators of this study have previously published the outcomes of ultrasound guided
15
16 433 laparoscopic ovarian wedge resection for the management of recurrent serous borderline ovarian
17
18 434 tumours⁽¹⁷⁾ and in the context of treatment for anti-NMDA receptor encephalitis.⁽²³⁾ Whilst both surgical
19
20 435 and oncological outcomes reported were successful, there was no measurable effect on the ovarian
21
22 436 reserve assessed post-operatively. This prospective trial therefore, will evaluate the effectiveness of this
23
24 437 method of FPS and provide real-time evidence for the post-operative effects on ovarian reserve. The
25
26 438 findings from the study will allow clinicians to provide informative counselling, so that women can
27
28 439 make well informed decisions regarding their future fertility before deciding to undergo surgery.
29
30 440 Furthermore, the technique is readily available and considered a low cost treatment option for the
31
32 441 management of benign ovarian cysts. Particularly, considering transvaginal ultrasound scanning is a
33
34 442 competency acquired by all UK trainees, and therefore many specialists already attain the skills to
35
36 443 implement this method of FPS. The procedure is therefore considered to be widely applicable
37
38 444 nationally.

39 445

40 446 **Author's contributions**

41 447 JY and LSK conceived and designed the study. LSK drafted the trial protocol. JY, JV and MEB
42
43 448 provided methodological and statistical expertise. JY and SGM provide expertise in clinical outcomes
44
45 449 following ultrasound. LSK and BPJ drafted the manuscript. LSK and SS with the support of the trial
46
47 450 Principal Investigator, have responsibilities for day-to-day running of the trial including participant
48
49 451 recruitment, data collection and liaising with other sites. All authors critically reviewed and approved
50
51 452 the final version of the manuscript.

52 453

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2
3 454 **Funding**

4
5 455 This research received no specific grant from any funding agency in the public, commercial or not-
6
7 456 for-profit sectors

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9 457

10
11 458 **Disclaimer**

12
13 459 The views expressed in this publication are those of the author (s)

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16
17 461 **Competing interests**

18
19 462 None declared.

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22
23 464 **Provenance and peer review**

24
25 465 Not commissioned; externally peer reviewed.

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27 466

28
29 467 **Figure and Table Legend**

30
31 468 Figure 1: Summary of Trial Design

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33 469 Table 2: Pilot data compared to control study

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35 470

36
37 471 **Trial Personnel**

38
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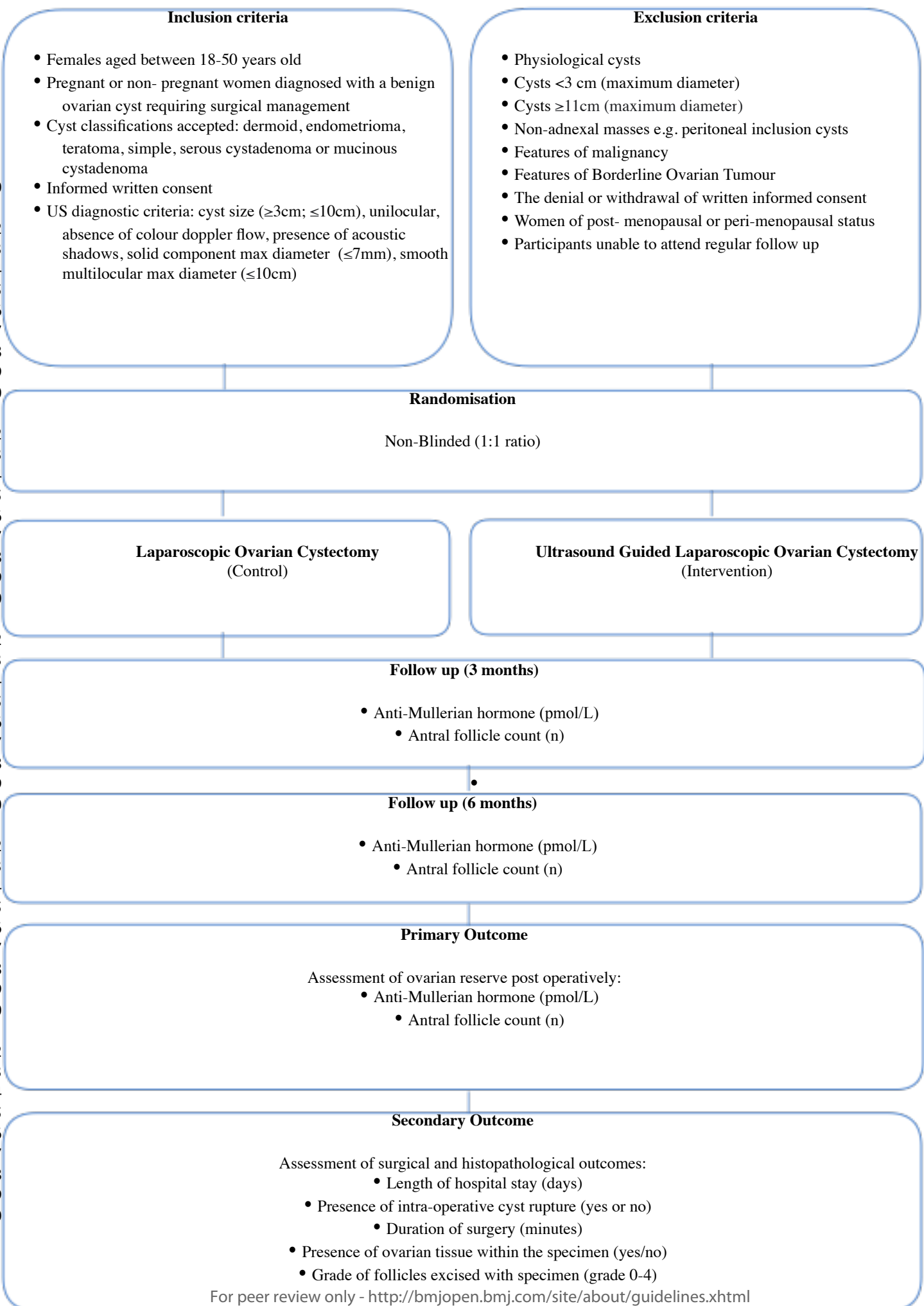
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For peer review only



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page/ (line number)
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1 (3-5)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2 (51)
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	All pages (footer)
Funding	4	Sources and types of financial, material, and other support	17(455-456)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 (9-16)
	5b	Name and contact information for the trial sponsor	19 (472)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4(85-141)
	6b	Explanation for choice of comparators	4(85-141)
Objectives	7	Specific objectives or hypotheses	6(143-161)

1				
2	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6 (165-199)
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8	Methods: Participants, interventions, and outcomes			
9				
10	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8 (208-236)
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14	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 (173-199)
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19	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10 (251-269)
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22		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11 (301-306)
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26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
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31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
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34	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6 (149-157)
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42	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1
43				
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47	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13 (342-379)
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51	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8 (208-217)
52				
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Methods: Assignment of interventions (for controlled trials)

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55				
56	Allocation:			Page 9
57				(226-249)
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2	Sequence	16a	Method of generating the allocation sequence (eg, computer-generated	Page 9
3	generation		random numbers), and list of any factors for stratification. To reduce	(226-249)
4			predictability of a random sequence, details of any planned restriction (eg,	
5			blocking) should be provided in a separate document that is unavailable to	
6			those who enrol participants or assign interventions	
7				
8				
9	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central	Page 9
10	concealment		telephone; sequentially numbered, opaque, sealed envelopes), describing	(226-249)
11	mechanism		any steps to conceal the sequence until interventions are assigned	
12				
13	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and	Page 9
14			who will assign participants to interventions	(226-249)
15				
16	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants,	Page 9
17			care providers, outcome assessors, data analysts), and how	(226-249)
18				
19		17b	If blinded, circumstances under which unblinding is permissible, and	Page 9
20			procedure for revealing a participant's allocated intervention during the trial	(226-249)
21				
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Methods: Data collection, management, and analysis

23				
24				
25	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other trial	Page 12
26	methods		data, including any related processes to promote data quality (eg, duplicate	(308-326)
27			measurements, training of assessors) and a description of study	
28			instruments (eg, questionnaires, laboratory tests) along with their reliability	
29			and validity, if known. Reference to where data collection forms can be	
30			found, if not in the protocol	
31				
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33		18b	Plans to promote participant retention and complete follow-up, including list	Page 15
34			of any outcome data to be collected for participants who discontinue or	(380-385)
35			deviate from intervention protocols	
36				
37	Data management	19	Plans for data entry, coding, security, and storage, including any related	Page 12
38			processes to promote data quality (eg, double data entry; range checks for	(308-326)
39			data values). Reference to where details of data management procedures	
40			can be found, if not in the protocol	
41				
42				
43	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes.	Page 15
44			Reference to where other details of the statistical analysis plan can be	(380-385)
45			found, if not in the protocol	
46				
47		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 15
48				(380-385)
49				
50		20c	Definition of analysis population relating to protocol non-adherence (eg, as	Page 15
51			randomised analysis), and any statistical methods to handle missing data	(380-385)
52			(eg, multiple imputation)	
53				
54				

Methods: Monitoring

1				
2	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Page 12 (308-326)
3				
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9		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13 (342-379)
10				
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13	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12 (328-340)
14				
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17	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12 (308-326)
18				
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21	Ethics and dissemination			
22				
23	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15 (387-394)
24				
25				
26	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	11 (301-306)
27				
28				
29				
30	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8 (208-217)
31				
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34		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
35				
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37	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12 (308-326)
38				
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42	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17(462)
43				
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45	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12 (308-326)
46				
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48	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12 (328-340)
49				
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51	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15 (388-394)
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57		31b	Authorship eligibility guidelines and any intended use of professional writers	17 (447-453)
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2 31c Plans, if any, for granting public access to the full protocol, participant-level NA
3 dataset, and statistical code
4

5 **Appendices**

6
7 Informed consent 32 Model consent form and other related documentation given to participants Appendix
8 materials and authorised surrogates
9

10 Biological specimens 33 Plans for collection, laboratory evaluation, and storage of biological NA
11 specimens for genetic or molecular analysis in the current trial and for
12 future use in ancillary studies, if applicable
13

14 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013
15 Explanation & Elaboration for important clarification on the items. Amendments to the
16 protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT
17 Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)"
18 license.
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