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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

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Abstract

Introduction: The lifetime risk of women undergoing surgery for the presence of benign ovarian pathology in the united kingdom (UK) is 5-10%.[1] Despite minimally invasive surgical techniques, evidence suggests a number of healthy ovarian follicles and tissues are resected intraoperatively, resulting in subsequent decline of ovarian reserve. Increasing demand for the implementation of fertility sparing surgical techniques is therefore prevalent. This study will evaluate the effect on ovarian reserve following two different surgical interventions for the management of benign ovarian cysts.

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

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

Trial registration number: NCT05032846

Key words

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

Strengths and limitations of this study

- This is the only reported prospective, randomised controlled trial to assess the use of intra-operative ultrasound as a method of fertility preservation surgery
- The trial will provide an evaluation of two different surgical interventions for the management of benign ovarian cysts, in order to optimise women's ovarian reserve
- The intervention is non- blinded
- Follow up of patients is limited to 6 months

Background

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

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

pathology is 5-10%,[1] it is perhaps understandable why an increasing demand for the implementation of fertility sparing surgical techniques for women with benign pathology is also prevalent.[1] Such demand is further exacerbated by the increasing age of motherhood observed over the last few decades.[8] Increasing age is associated with poorer oocyte quality and yield, thereby inadvertently increasing the risk of involuntary childlessness as a direct consequence of age related fertility decline.[9] If women delay attempting pregnancy to a later age, in addition to the risks of surgically induced impairment of ovarian tissue, overall chances of achieving pregnancy in the future maybe significantly reduced. It is imperative therefore, that fertility sparing techniques are implemented, where possible, in women of reproductive age in order to optimize the chances of future successful conception.

Intra-operative ultrasound

The use of intraoperative ultrasound has been widely implemented amongst specialties.[10] However, within gynaecological surgery, it is not as commonly recognized, despite evidence that it can be used as an adjunct to improving minimally invasive surgical techniques.[11] This is primarily due to the improved visualization of the operative field, which can assist more technically difficult surgical procedures, thus minimizing intraoperative complications.[12] The application of ultrasound guidance within gynaecological procedures have included predominantly ovarian cyst aspiration, in vitro fertilization and removal or insertion of intra uterine devices.[10] Although pre-operative imaging provides procedural planning, it cannot compare to the information gained from real time imaging. For example, in previous studies, intraoperative ultrasound detected more myomas during myomectomy than pre-operative transvaginal imaging.[13] Furthermore, it provides the potential to assess lesion margins, ensuring resection of pathology is complete with negligible damage to surrounding healthy tissues.[11] This is consistent with a recent systematic review, which also demonstrated that albeit a novel technique, amongst various case series, pathology can be safely resected without incurring injury to healthy reproductive tissue.[11] Therefore, intraoperative ultrasound has the potential to improve surgical accuracy, reduce complications and improve patient safety. The application of intraoperative ultrasound as an adjunct to FSS has not been widely

researched, with only a few case series reporting surgical outcomes on patients undergoing treatment for pre-malignant or malignant pathology.[14]

Aim

To evaluate the effect on ovarian reserve following two different surgical interventions for the management of benign ovarian cysts.

Primary objective

To determine the efficacy of intra-operative ultrasound as a method of fertility preservation surgery.

Secondary objectives

To determine the surgical and histopathological outcomes in women who have undergone intraoperative ultrasound guided ovarian cystectomy and compare to the control group.

Hypothesis

Intra-operative ultrasound guided laparoscopic ovarian cystectomy will preserve more healthy ovarian tissue, resulting in a significantly reduced decline of ovarian reserve observed at 3 and 6 months post operatively, as measured by AMH and antral follicle count (AFC), when compared to laparoscopic ovarian cystectomy.

Methods

Trial Design

This is a multi-centre randomised controlled trial with two parallel arms, comparing laparoscopic ovarian cystectomy with ultrasound guided laparoscopic ovarian cystectomy (UGLOC) for the management of benign ovarian cysts. Participants will be recruited from two tertiary gynaecology centres in the UK. Participants will be followed up at 3 and 6 months post operatively to assess

markers of ovarian reserve. Recruitment will commence October 2021, with follow up and assessment expected to conclude in October 2024. **Figure 1** summarises the trial design.

Inclusion criteria

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

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

- Cyst size ≥3cm; ≤10cm
- International Ovarian Tumour Analysis Benign features (IOTA B) present:
    - Unilocular
    - Solid components (largest diameter ≤7mm)
    - Acoustic shadows
    - No blood flow
    - Smooth multilocular cyst (largest diameter ≤10cm)

Exclusion criteria

- Cysts deemed to be clearly physiological and <3 cm in maximum diameter
- Cysts ≥11cm in maximum diameter
- Non-adnexal masses e.g. peritoneal inclusion cysts
- Cyst with features of malignancy
- The denial or withdrawal of written informed consent

- Women of post- menopausal or peri-menopausal status
- Participants unable to attend regular follow up

Outcome measures

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

Enrolment

All women referred to the outpatient gynecology clinic with a suspected ovarian cyst will have a pelvic transvaginal ultrasound scan (2D and 3D ultrasonography) as part of routine clinical care. If an ovarian cyst is observed on ultrasound, it will be assessed according to local protocols based on simple descriptors and international ovarian tumor analysis (IOTA) simple rules. Depending on the severity of symptoms, nature of the cyst and whether surgical management is indicated to treat; should the woman fulfil aspects of the inclusion criteria, she will be invited to participate in the study.

The study co-ordinator will be responsible for managing the registration of each participant to the trial and their allocation to either treatment arm. All participants will sign a written consent form, witnessed by a member of the research team, at least 24 hours after the participant information sheet has been read. All consent forms will be scanned into the electronic medical notes. It will not be possible to carry out any tasks pertaining to the trial, until written consent from the participant has been obtained.

Randomisation

A separate research team within Imperial College London Healthcare Trust, Department of Cancer and Surgery will be responsible for the allocation process, by producing randomisation sealed

envelopes in a ratio of 1:1. The team will be asked to print labels with the allocated group and fold them so the content cannot be seen. They will then give the folded labels back to the study co-ordinator, who will be asked to place them into sealed envelopes, which will be numbered chronologically. The co-ordinator will not be able to see the content of the labels, in order to ensure concealment. Given that it is necessary for the surgeon to know which operation to perform, both the participant and research team will not be blinded. The allocated arm of the RCT will be recorded on the Trial Subject Enrolment Log. The Principal Investigator (PI) will be responsible for keeping the randomisation list.

Procedures

During the recruitment process, once consent to participate in the trial has been obtained, a member of the research team will select a sealed randomisation envelope as numbered chronologically, which will then assign the patient to a treatment arm. The chronological order of envelopes will prevent the member of the research team performing the randomisation themselves, or from selecting another envelope, should they be dissatisfied with the treatment arm assigned to the participant. The participant will then undergo a blood test to record their baseline pre-operative AMH level. The same surgeons will operate on women, regardless of treatment arm assigned. This will aim to exclude bias, which may otherwise attribute findings to the surgeon operating. Surgery will be performed at Imperial Healthcare NHS Trusts and at the University College London Hospitals NHS Foundation Trust by an experienced clinician. All participants will attend pre-operative assessment with an anesthetist as standard practice of care. In most cases, the surgical procedure is performed either as a day case or overnight stay.

Surgical Intervention

Laparoscopic ovarian cystectomy (control)

Participants will undergo a laparoscopic ovarian cystectomy in the absence of ultrasound guidance. Surgery will be performed according to basic laparoscopy principles from the British Society of Gynaecological Endoscopy (BSGE).

Ultrasound guided laparoscopic ovarian cystectomy (intervention)

Participants will undergo an ultrasound guided laparoscopic ovarian cystectomy. An assistant with competencies in pelvic ultrasound will insert a transrectal probe intraoperatively, and provide real-time ultrasound images of the ovary and cyst to be resected. The continuous use of intra-operative ultrasound ensures the surgeon is able to differentiate between healthy ovarian tissue and cyst content. It is often necessary following peritoneal insufflation to infiltrate 500mL of normal saline (0.9%) into the pelvis to enhance the ultrasound image quality and transmission of the ultrasound. The surgeon will then perform the laparoscopic ovarian cystectomy under ultrasound guidance, following basic laparoscopy principles from the BSGE.

Follow up

Post operatively, patients will return to the outpatient gynaecology clinic for follow up at 3 and 6 months, whereby the AMH level will be checked. The blood samples will be processed by Imperial College Healthcare Trust laboratories or University College London Hospitals NHS Foundation Trust, depending on the site taken, and will be discarded as per local protocol once the AMH has been determined. There are no specific storage or transfer requirements outside of normal practice. In addition, a transvaginal ultrasound scan will be performed during the follow up appointment, to measure AFC and assess volume of preserved ovarian tissue. Following the second follow up attendance at 6 months, no further input is required from the participant in the study.

Discontinuation or withdrawal of participants

Participants who give consent to their recruitment to the trial agree to the intervention, compliance with follow up assessments and data collection. Participants are free to withdraw at any time from the protocol treatment without reason. Furthermore, participants may be withdrawn from the trial by members of the research team, should participation in the trial no longer be deemed within the participant's best interest. All data captured in relation to their participation may be destroyed at their

request. Any decision to withdraw a participant from the study will be recorded in the electronic clinical record (eCRF) files and medical notes. Reasons for stopping the trial include: non-compliance by members of the research team to adherence of the study protocol, participants withdrawing consent or adverse outcomes reported following intervention.

Patient and public involvement

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

Modification of the protocol

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

Data and trial management

Electronic clinical record files (eCRFs) have been designed to assist data collection. Members of the research team will be responsible for the completion of the eCRFs, whereby the PI will ensure accuracy of all data reported. Members of the research team and all aspects of the study protocol will adhere to the principles of the General Data Protection Regulation (2016/679) and the Data Protection Act (2018). All personal data will be password protected and held on a database, accessible only from a registered NHS Trust computer. Following data collection, all information will be anonymized during the data analysis stage, whereby access will be restricted to the PI and study coordinator only.

The sponsor of the study reserves the right to store all anonymized data for 10 years after the study has finished, in relation to data subject consent forms and ten years after the study has completed in relation to primary research data. Following this, the sponsor will adhere to the confidential information trust destruction procedures for disposal of data. A trial management group (TMG) has been designed including the PI, two study co-ordinators and trial staff. They are responsible for the day to day running of the trial. The TMG will meet every 6 weeks to discuss recruitment numbers, adverse events (AEs) encountered or potential amendments to the study protocol, if required. The study may be subject to inspection and audit by the sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research for Health and Social Care. Direct access to source data/documents as requested will be permitted. A data safety monitoring board is not deemed necessary, as the study is associated with extremely low risks.

Safety

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

- '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.

Reports of related and unexpected SAEs will be submitted within 15 days of the PI becoming aware of the event, using the NRES SAE form for non-IMP studies. The PI will notify the Sponsor of all related and unexpected SAEs.

Sample size and power calculation

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

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

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

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

Sample Size Calculation AMH Differences (pmol/L)		Treatment Group	Control Group		
		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	

The minimum sample size is 11 patients. Considering the small size of the pilot and the very low risks thus far, we have included 2 more observations to boost power, and assume an attrition of 25%. This leads to a total of 16 participants per arm, with a power of 0.95 to detect a difference at the 5% level using a standard t-test on the log-transformed difference in means of AMH; our robust proxy for fertility. Thus, using the pilot data, we anticipate 32 participants will need to be recruited to demonstrate differential AMH levels (**Table 2).** It will be necessary to perform an interim analysis, given the low number of participants available for the power calculation.

Table 2: Power Calculation

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

Secondary Analysis

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

Ethics and Dissemination

This study will be conducted in accordance with the principles of Good Clinical Practice. This protocol was submitted to the Health Research Authority and Chelsea Research Ethics Committee, whereby a favourable ethical opinion was granted. The reference number is 21/LO/036. Subsequent approval by individual ethical committee and competent authority was granted. Any modification to the protocol will be updated on the ClinicalTrials.gov website and disseminated to all relevant parties

(NCT05032846). The results will be published in peer-reviewed journals and disseminated at national and international conferences.

Data sharing

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

Discussion

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

Recent advancements within ultrasound technology have facilitated the enhancement in diagnostic accuracy, as evidenced by the detection of smaller ovarian lesions or pathology on ultrasound scan.[14] Furthermore, the ability to delineate cyst content from healthy ovarian tissue also reduces the risk of cyst rupture; which depending on the content may have detrimental effects on the pelvis. Considering evidence suggests a significantly higher volume of normal ovarian tissue is resected during cystectomy of endometriomas when evaluated histologically,[17] the ability to preserve optimal healthy ovarian tissue is also a benefit of intra-operative ultrasound. Therefore, combining this diagnostic tool with FSS, provides an apt alternative to the otherwise *blind* resection of healthy ovarian tissue during ovarian cystectomy.

The PI and co-investigators of this study have previously published the outcomes of ultrasound guided laparoscopic ovarian wedge resection for the management of recurrent serous borderline ovarian tumours[14] and in the context of treatment for anti-NMDA receptor encephalitis.[18] Whilst both surgical and oncological outcomes reported were successful, there was no measurable effect on the ovarian reserve assessed post-operatively. This prospective trial therefore, will evaluate the efficacy of this method of FSS and provide real-time evidence for the post-operative effects on ovarian reserve. In addition, findings from the study will be able to deduce whether the type of ovarian cyst resected is associated with loss of ovarian reserve, allowing clinicians to provide informative counselling, so that women can make well informed decisions regarding their future fertility before deciding to undergo surgery. Furthermore, the technique is readily available and considered a low cost treatment option for the management of benign ovarian cysts. Particularly, considering transvaginal ultrasound scanning is a competency acquired by all UK trainees, and therefore many specialists already attain the skills to implement this method of FSS. The procedure is therefore considered to be widely applicable nationally.

Author's contributions

JY and LSK conceived and designed the study. LSK drafted the trial protocol. JY, JV and MEB provided methodological and statistical expertise. JY and SGM provide expertise in clinical outcomes following ultrasound. LSK and BPJ drafted the manuscript. LSK and SS with the support of the trial Principal Investigator, have responsibilities for day-to-day running of the trial including participant recruitment, data collection and liaising with other sites. All authors critically reviewed and approved the final version of the manuscript.

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Disclaimer

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

Competing interests

None declared.

Provenance and peer review

Not commissioned; externally peer reviewed.

Trial Personnel

See **Table 3.**

Table 3: Trial Personnel

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Co-investigator (s)	**Miss Lorraine Kasaven** Clinical Research Fellow Imperial College London Department of Cancer and Surgery l.kasaven@nhs.net **Mr Srdjan Saso** Consultant Gynaecological Oncology Surgeon Imperial College London Healthcare NHS Trust Srdjan.saso01@imperial.ac.uk **Mr Benjamin P Jones** Obstetrics and Gynaecology Trainee Imperial College London Healthcare NHS Trust Benjamin.jones@nhs.net **Professor Sadaf Ghaem-Maghami** Consultant Gynaecological Oncology Surgeon Imperial College London Healthcare NHS Trust s.ghaem-maghami@imperial.ac.uk
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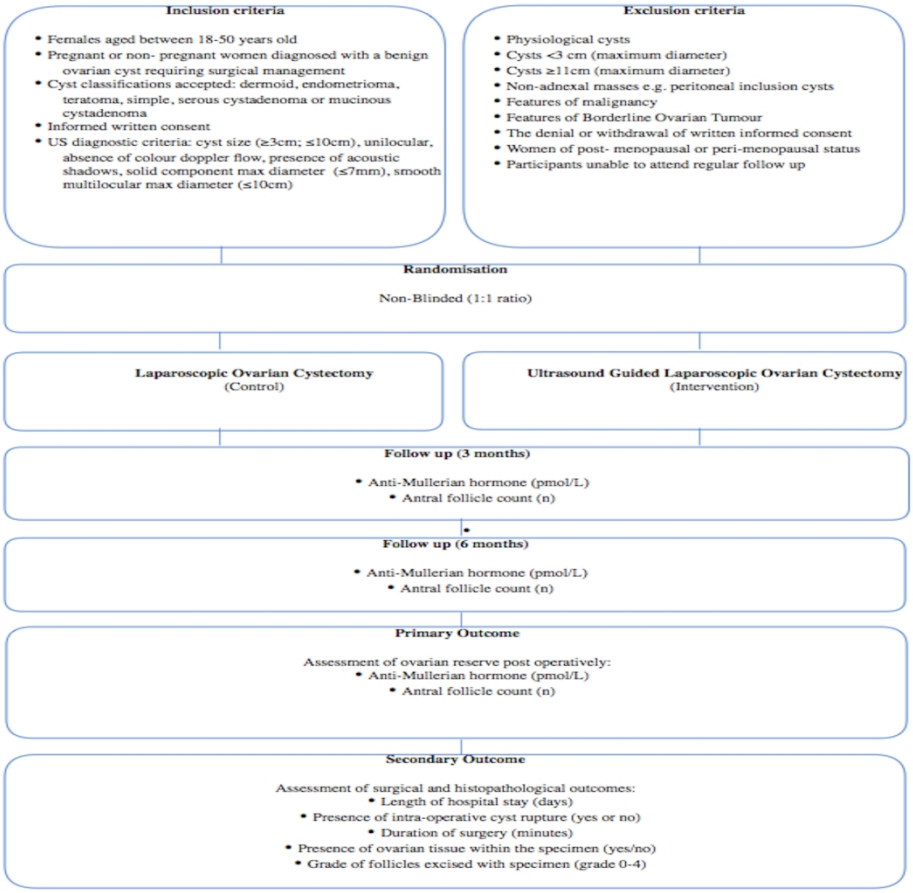
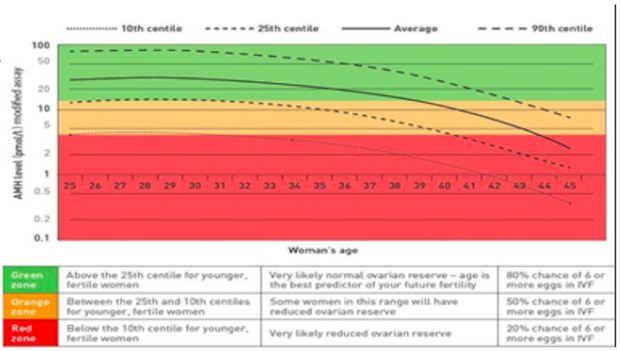


89x89mm (600 x 600 DPI)

Figure 2:

Normed percentile transformation of Anti-Mullerian hormone (AMH) values



Green zone	Above the 25th centile for younger, fertile women	Very likely normal ovarian reserve – age is the best predictor of your future fertility	80% chance of 6 or more eggs in IVF
Orange zone	Between the 25th and 10th centiles for younger, fertile women	Some women in this range will have reduced ovarian reserve	50% chance of 6 or more eggs in IVF
Red zone	Below the 10th centile for younger, fertile women	Very likely reduced ovarian reserve	20% chance of 6 or more eggs in IVF

90x90mm (300 x 300 DPI)

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

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

*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 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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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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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Abstract

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

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

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

Trial registration number: NCT05032846

Key words

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

Strengths and limitations of this study

- This is the only reported prospective, randomised controlled trial to assess the use of intra-operative ultrasound as a method of fertility preservation surgery
- The trial will provide an evaluation of two different surgical interventions for the management of benign ovarian cysts, in order to optimise women's ovarian reserve
- The intervention is non-blinded
- Follow up of women is limited to 6 months

Background

Within the field of reproductive medicine, advancements over the last few decades have facilitated the rapidly emerging area of expertise, referred to as fertility preservation. This includes various methods to preserve reproductive tissue or gametes such as medical, surgical or laboratory techniques, thus empowering women to preserve their fecundity with a view to achieving pregnancy at a later date.[1] Such techniques were initially considered for women of reproductive age diagnosed with cancer, embarking on gonadotoxic treatment regimens including chemotherapy or radiotherapy, or undergoing radical surgery to remove gynaecological organs, thus rendering them infertile. Thus, in the context of surgical management of gynaecological cancers, there has been an increasing demand for less radical procedures, with a shift towards conservative surgical methods, in order to preserve the reproductive organs. In appropriately selected women, this enables the opportunity to balance the risks of recurrence from disease, whilst reserving the ability to conceive in the future. As such, the mainstay treatment of Borderline Ovarian Tumours for example, in women with early-stage disease, non-invasive implants or for those who wish to conceive, is fertility preservation surgery (FPS). Such procedures include performing a unilateral salpingo-oophorectomy (USO) or ovarian cystectomy, compared to previously adopted surgical methods of radical debulking, which required bilateral salpingo-oophorectomy. Evidence suggests that in this context, FPS is both safe and feasible.[2]

Consideration of fertility however, is no longer limited to women undergoing treatment for cancer, as evidence suggests 1 in 6 women now experience infertility.[3] Although there are a number of causes, it is also prevalent amongst women diagnosed with benign pathology, such as endometriosis or ovarian cysts. Infertility can be caused either by the underlying pathology itself, or indirectly associated with the surgical intervention required to treat.[4] The latter is attributed to the fact that ovarian surgery, despite minimally invasive techniques, results in the resection of a number of healthy ovarian follicles and tissue.[5] This is exemplified from a study demonstrating that anti-Mullerian hormone (AMH), a reliable marker of ovarian reserve, is reduced post-operatively following surgery for endometriosis.[6] Considering the lifetime risk of women undergoing surgery for the presence of benign ovarian pathology is 5-10%,[7] it is perhaps understandable why there is an increasing demand

for fertility preserving surgical techniques for women with benign pathology.[8] Such demand is further exacerbated by the increasing age of motherhood observed over the last few decades.[9] Increasing age is associated with poorer oocyte quality and yield, thereby inadvertently increasing the risk of involuntary childlessness as a direct consequence of age related fertility decline.[10] If women delay attempting pregnancy to a later age, in addition to the risks of surgically induced impairment of ovarian tissue, overall chances of achieving pregnancy in the future maybe significantly reduced. It is imperative therefore, that fertility preserving techniques are implemented, where possible, in women of reproductive age in order to optimize the chances of future successful conception.

Intra-operative ultrasound

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

only a few case series reporting surgical outcomes on patients undergoing treatment for pre-malignant or malignant pathology.[17]

Aim

To compare the effect of two different surgical interventions, including either laparoscopic ovarian cystectomy (control group) or ultrasound guided laparoscopic ovarian cystectomy (UGLOC) (experimental group) for the management of benign ovarian cysts, on the ovarian reserve measured 3 and 6 months post operatively.

Primary objective

To compare the difference in serum AMH level and AFC number at 3 and 6 months post operatively in women who have undergone UGLOC and compare to the control group.

Secondary objectives

Secondary outcomes include assessment of various surgical and histopathological outcomes including: duration of hospital stay (days), duration of surgery (mins), presence of intra-operative cyst rupture (yes/no), presence of ovarian tissue within the specimen (yes/no) and the grade of follicles excised with specimen (grade 0-4).

Hypothesis

The difference in serum AMH level and AFC number measured at 3 and 6 months post operatively will be significantly less following UGLOC when compared to laparoscopic ovarian cystectomy.

Methods

Trial Design

This is a single-centre randomised controlled trial with two parallel arms, comparing laparoscopic ovarian cystectomy with UGLOC for the management of benign ovarian cysts. Women will be

recruited from one tertiary gynaecology centre in the UK. They will be followed up at 3 and 6 months post operatively to assess markers of ovarian reserve. Recruitment will commence from October 2021, with follow up and assessment expected to conclude in October 2024. **Figure 1** summarises the trial design.

Inclusion criteria

- Women aged between 18-45 years old
- Non- pregnant women diagnosed with a benign ovarian cyst requiring surgical management
- Cyst classifications accepted: mature teratoma (dermoid), simple cyst, serous cystadenoma or mucinous cystadenoma
- Informed written consent

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

- Cyst size ≥3cm; ≤10cm
- International Ovarian Tumour Analysis Benign features (IOTA B) present:
    - Unilocular
    - Solid components (largest diameter ≤7mm)
    - Acoustic shadows
    - No blood flow
    - Smooth multilocular cyst (largest diameter ≤10cm)

Exclusion criteria

- Cysts deemed to be clearly physiological and <3 cm in maximum diameter
- Cysts ≥11cm in maximum diameter
- Bilateral ovarian cysts
- Non-adnexal masses e.g. peritoneal inclusion cysts
- Cyst with features of malignancy

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

Outcome measures

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

Enrolment

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

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

has been read. All consent forms will be scanned into the electronic medical notes. It will not be possible to carry out any tasks pertaining to the trial, until written consent from the participant has been obtained.

Randomisation

A separate research team within Imperial College London Healthcare Trust, Department of Cancer and Surgery will be responsible for the allocation process, by producing randomisation sealed envelopes in a ratio of 1:1. The team will be asked to print labels with the allocated group and fold them so the content cannot be seen. They will then give the folded labels back to the study co-ordinator, who will be asked to place them into opaque sealed envelopes, which will be numbered in ascending order. The co-ordinator will not be able to see the content of the labels, in order to ensure concealment. Given that it is necessary for the surgeon to know which operation to perform, both the participant and research team will not be blinded. The allocated arm of the RCT will be recorded on the Trial Subject Enrolment Log. The Principal Investigator (PI) will be responsible for keeping the randomisation list.

Procedures

During the recruitment process, once consent to participate in the trial has been obtained, a member of the research team will select a sealed randomisation envelope as numbered in ascending order, which will then assign the particpant to a treatment arm. The ascending order of envelopes will prevent the member of the research team performing the randomisation themselves, or from selecting another envelope, should they be dissatisfied with the treatment arm assigned to the participant. The participant will then undergo a blood test to record their baseline pre-operative AMH level. The same surgeons will operate on all participants, regardless of treatment arm assigned. This will aim to exclude bias, which may otherwise attribute findings to the surgeon operating. Surgery will be performed at Imperial Healthcare NHS Trusts by an experienced clinician. All participants will attend pre-operative assessment with an anesthetist as standard practice of care. All women undergoing surgery will be required to have an overnight stay in hospital.

Surgical Intervention

Laparoscopic ovarian cystectomy (control)

Laparoscopic entry will be performed according to basic laparoscopic principles from the British Society of Gynaecological Endoscopy (BSGE).[7] Pneumoperitoneum is achieved through infiltration of carbon dioxide into the pelvis, which provides insufflation and visualization of the pelvic organs. Participants will undergo laparoscopic ovarian cystectomy in the absence of ultrasound guidance. Following removal of the cyst through laparoscopic specimen retrieval bags, routine closure is performed.

Ultrasound guided laparoscopic ovarian cystectomy (intervention)

Laparoscopic entry and peritoneal insufflation of carbon dioxide are performed to achieve pneumoperitoneum. Following laparoscopic entry and assessment of the operating field, 500mLs of normal saline (0.9%) is infiltrated into the pelvis, for enhancement of the ultrasound image quality and transmission of the ultrasound. This remains within the pelvis during the course of the operation. An assistant with competencies in pelvic ultrasound scanning will insert a transrectal probe intraoperatively, and provide real-time ultrasound images of the ovary and cyst to be resected. A non-traumatic instrument is used to locate the cyst, whilst correlating between the laparoscopic and ultrasound images. The cystectomy is performed under continuous ultrasound guidance, above the level of the saline solution, ensuring the surgeon is able to differentiate between healthy ovarian tissue and cyst content. Following removal of the cyst through laparoscopic specimen retrieval bags, routine closure is performed.

Follow up

Post operatively, participants will return to the outpatient gynaecology clinic for follow up at 3 and 6 months, whereby the AMH level will be assessed. The blood samples will be processed by Imperial College Healthcare Trust laboratories and will be discarded as per local protocol once the AMH has been determined. There are no specific storage or transfer requirements outside of normal practice. In addition, a transvaginal ultrasound scan will be performed during the follow up appointment, to

measure AFC and assess volume of preserved ovarian tissue. Following the second follow up attendance at 6 months, no further input is required from the participant in the study.

Discontinuation or withdrawal of participants

Participants who give consent to their recruitment to the trial agree to the intervention, compliance with follow up assessments and data collection. Participants are free to withdraw at any time from the protocol treatment without reason. Furthermore, participants may be withdrawn from the trial by members of the research team, should participation in the trial no longer be deemed within the participant's best interest. All data captured in relation to their participation may be destroyed at their request. Any decision to withdraw a participant from the study will be recorded in the electronic clinical record (eCRF) files and medical notes. Reasons for stopping the trial include: non-compliance by members of the research team to adherence of the study protocol, participants withdrawing consent or adverse outcomes reported following intervention.

Patient and public involvement

Ten women were approached by members of the research team from outpatient Gynaecology clinics, to request their assistance reviewing participant information resources applicable to the study. If agreeable, they were provided with a copy of the patient information leaflet and consent form to review over the course of a week. They then voluntarily attended a virtual *research focus* meeting, whereby their feedback regarding aspects of the study protocol were sought including: feasibility and acceptability of the study design, methods of monitoring ovarian reserve post operatively and acceptability of the intervention arm of the trial. They also participated in revising patient information leaflets and GP letter templates.

Modification of the protocol

Any amendments to the research protocol will be firstly agreed by the Principle Investigator (PI) and the study coordinators. This may include aspects of the study design, participant recruitment, sample

size, interventions or ethics documents including participant information sheet or consent form. Implementation of changes made will depend on subsequent approval from the Research Ethics Committee. Any amendments made will be updated on the clinialtrials.gov website accordingly.

Data and trial management

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

Safety

Any questions concerning adverse event reporting will be directed to the PI in the first instance. For non-serious AEs, whether expected or not, a brief description of associated clinical symptoms with date and duration of onset will be documented in the medical notes. For SAEs**,** an SAE form will be

completed and emailed to the PI within 24 hours. Specifically, relapse and death due to other pathology, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. All SAEs will be reported to the Chelsea Research and Ethics Committee where in the opinion of the PI, the event was:

AMH Differences (pmol/L)

- '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.

Reports of related and unexpected SAEs will be submitted within 15 days of the PI becoming aware of the event, using the NRES SAE form for non-IMP studies. The PI will notify the Sponsor of all related and unexpected SAEs.

Sample size and power calculation

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

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

(Table 1)

	Treatment Group (Pilot)	Control Group (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

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

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

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

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

Secondary Analysis

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

Ethics and Dissemination

This study will be conducted in accordance with the principles of Good Clinical Practice. This protocol was submitted to the Health Research Authority and Chelsea Research Ethics Committee, whereby a favourable ethical opinion was granted. The reference number is 21/LO/036. Subsequent approval by an individual ethical committee and competent authority was granted. Any modification to the protocol will be updated on the ClinicalTrials.gov website and disseminated to all relevant parties (NCT05032846). The results will be published in peer-reviewed journals and disseminated at national and international conferences.

Data sharing

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

Potential biases within the study

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

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

Discussion

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

Recent advancements within ultrasound technology have facilitated the enhancement in diagnostic accuracy, as evidenced by the detection of smaller ovarian lesions or pathology on ultrasound scan.[17]

Furthermore, the ability to delineate cyst content from healthy ovarian tissue also reduces the risk of cyst rupture; which depending on the contents may have detrimental effects on the pelvis. The ability to preserve optimal healthy ovarian tissue is therefore a benefit of intra-operative ultrasound, providing an apt alternative to the otherwise *blind* resection of healthy ovarian tissue during ovarian cystectomy.

The PI and co-investigators of this study have previously published the outcomes of ultrasound guided laparoscopic ovarian wedge resection for the management of recurrent serous borderline ovarian tumours[17] and in the context of treatment for anti-NMDA receptor encephalitis.[23] Whilst both surgical and oncological outcomes reported were successful, there was no measurable effect on the ovarian reserve assessed post-operatively. This prospective trial therefore, will evaluate the effectiveness of this method of FPS and provide real-time evidence for the post-operative effects on ovarian reserve. The findings from the study will allow clinicians to provide informative counselling, so that women can make well informed decisions regarding their future fertility before deciding to undergo surgery. Furthermore, the technique is readily available and considered a low cost treatment option for the management of benign ovarian cysts. Particularly, considering transvaginal ultrasound scanning is a competency acquired by all UK trainees, and therefore many specialists already attain the skills to implement this method of FPS. The procedure is therefore considered to be widely applicable nationally.

Author's contributions

JY and LSK conceived and designed the study. LSK drafted the trial protocol. JY, JV and MEB provided methodological and statistical expertise. JY and SGM provide expertise in clinical outcomes following ultrasound. LSK and BPJ drafted the manuscript. LSK and SS with the support of the trial Principal Investigator, have responsibilities for day-to-day running of the trial including participant recruitment, data collection and liaising with other sites. All authors critically reviewed and approved the final version of the manuscript.

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Disclaimer

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

Competing interests

None declared.

Provenance and peer review

Not commissioned; externally peer reviewed.

Figure and Table Legend

Figure 1: Summary of Trial Design

Table 2: Pilot data compared to control study

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Inclusion criteria

- Females aged between 18-50 years old
- Pregnant or non- pregnant women diagnosed with a benign ovarian cyst requiring surgical management
- Cyst classifications accepted: dermoid, endometrioma, teratoma, simple, serous cystadenoma or mucinous cystadenoma
- Informed written consent
- US diagnostic criteria: cyst size (≥3cm; ≤10cm), unilocular, absence of colour doppler flow, presence of acoustic shadows, solid component max diameter (≤7mm), smooth multilocular max diameter (≤10cm)

Exclusion criteria

- Physiological cysts
- Cysts <3 cm (maximum diameter)
- Cysts ≥11cm (maximum diameter)
- Non-adnexal masses e.g. peritoneal inclusion cysts
- Features of malignancy
- Features of Borderline Ovarian Tumour
- The denial or withdrawal of written informed consent
- Women of post- menopausal or peri-menopausal status
- Participants unable to attend regular follow up

Randomisation

Non-Blinded (1:1 ratio)

Laparoscopic Ovarian Cystectomy
(Control)

Ultrasound Guided Laparoscopic Ovarian Cystectomy
(Intervention)

Follow up (3 months)

- Anti-Mullerian hormone (pmol/L)
- Antral follicle count (n)

Follow up (6 months)

- Anti-Mullerian hormone (pmol/L)
- Antral follicle count (n)

Primary Outcome

Assessment of ovarian reserve post operatively:

- Anti-Mullerian hormone (pmol/L)
- Antral follicle count (n)

Secondary Outcome

Assessment of surgical and histopathological outcomes:

- Length of hospital stay (days)
- Presence of intra-operative cyst rupture (yes or no)
- Duration of surgery (minutes)
- Presence of ovarian tissue within the specimen (yes/no)
- Grade of follicles excised with specimen (grade 0-4)

SPIRIT

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)

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)
Methods: Participants, interventions, and outcomes			
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)
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)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10 (251-269)
	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)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
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)
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
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)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8 (208-217)
Methods: Assignment of interventions (for controlled trials)			
Allocation:			Page 9 (226-249)

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 9 (226-249)
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 9 (226-249)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 9 (226-249)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 9 (226-249)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Page 9 (226-249)
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Page 12 (308-326)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Page 15 (380-385)
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Page 12 (308-326)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 15 (380-385)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 15 (380-385)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Page 15 (380-385)
Methods: Monitoring			

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)
	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)
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)
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)
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15 (387-394)
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)
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)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17(462)
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)
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)
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)
	31b	Authorship eligibility guidelines and any intended use of professional writers	17 (447-453)

	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

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