

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Kasaven, Lorraine S; Jones, Benjamin; Ghaem-Maghani, Sadaf; Verbakel, Jan; El-Bahrawy, Mona; Saso, Srdjan; Yazbek, Joseph

VERSION 1 – REVIEW

REVIEWER	Al Wattar, Bassel Birmingham Women's Hospital, Obstetrics and Gynaecology
REVIEW RETURNED	29-Dec-2021

GENERAL COMMENTS	<p>Thank you for asking me to review this protocol for a proposed randomised trial evaluating the benefits of using intra-operative transrectal ultrasound scanning (USS) to guide laparoscopic cystectomy.</p> <p>The proposal is bold and well thought, I applaud the authors for planning this study. Overall, I support the publication of this work, however, I have the following comments to clarify some elements of the submitted protocol: Major comments:</p> <p>1- From a philosophical point, I think it is important to specify if you are really doing a “fertility preservation surgical technique” or you are proposing a “diagnostic adjunct” to an established surgical technique. You are not really changing the surgical course, but rather, changing the tools supporting surgical decision making.</p> <p>2- I think it is important you implement and describe a standardised protocol for intra-operative scanning. Cannot have saline injected in some patients and not injected in others as this will increase performance bias especially since your sample size is small.</p> <p>3- I tried to replicate your sample size calculation, but I failed (Table 2 is bit missed up in the PDF so could not follow the numbers). To have a high degree of certitude evaluating effectiveness (I.E if USS works in real world), I find having 16 patients in each arm too small to cover the variation in baseline characteristics that could affect your outcomes (high bmi, low ovarian reserve at baseline, pregnancy, adhesions, etc..). I appreciate doing surgical RCTs is tough (been there done that). Has this calculation been adjusted for variation in patient characteristics? Maybe worth seeking an opinion from a statistician with trial expertise.</p> <p>4- I am a bit sceptical of your inclusion criteria for the following reasons:</p>
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	<p>a. The age range presented is too wide (up to 50 years old!). Invariably some of these 32 women will be peri-menopausal at/around 50 (an exclusion criterion set by you later).</p> <p>b. Also, women aged 45+ will have very little variations in their AMH purely due to poor ovarian reserve and it will skew your findings towards the null.</p> <p>c. Finally, why include pregnant women! GA has an effect on the baby and usually would not operate on a benign cyst in pregnancy unless it is complicated (e.g. Torsion).</p> <p>d. The last item says (bilateral cysts): does this mean you won't include those with unilateral cyst? Why so?</p> <p>Minor (stylistic) comments:</p> <p>1- The Abstract should not contain references</p> <p>2- The introduction is far too long. It reads like a chapter in a dissertation. For benefit of BMJ Open I think it can be shortened and made more concise.</p> <p>3- Ln115: I disagree that (fertility preservation) is a subspeciality on its own. Its an area or topic of expertise</p> <p>4- Ln131: I think you mean fertility rather than infertility?</p> <p>5- (why an increasing demand for the implementation of fertility sparing surgical techniques for women with benign pathology is also prevalent): increasing and prevalent is kind of a double positive in one sentence.</p> <p>6- There is so much confusion in the use of the terms: participants, patients, women, females throughout the manuscript: please standardise</p> <p>7- Similarly there is so much confusion with using (Fertility Sparing) and (fertility preservation) interchangeably, please standardise</p> <p>8- (Therefore, intraoperative ultrasound has the potential to improve surgical accuracy, reduce complications and improve patient safety.): making this argument in the introduction feels like you already make the conclusion before doing the study. I am not sure it increases safety, arguably introducing an additional (experimental) element to surgery in untrained hands can increase risk of complications (e.g. bowel injury).</p> <p>9- The aim/hypothesis/objectives are very confusing and need revision:</p> <p>a. Ln172: To evaluate the effect of what?</p> <p>b. Ln176: (intra-operative ultrasound as a method of fertility preservation surgery): first time your present ultrasound as a fertility preservation surgery "method". Not sure if actually ultrasound can be defined on its own as a surgical method</p> <p>c. Please be mindful of the use of efficacy vs effectiveness</p> <p>10- How do you plan to define (peri-menopausal women)?</p> <p>11- Please specify that the randomisation envelopes are opaque</p> <p>12- Is chronologically the correct word? I think they are in ascending order, but not sure of the element of time here. Ill leave this to the editors to judge.</p> <p>13- Ln270: I think the length of hospital stay must be standardised or it will affect the accuracy of this secondary outcome.</p> <p>14- Ln276: Need Reference for BSGE</p> <p>15- I think it is better to describe how the concomitant use of ultrasound intraoperatively will improve tissue handling and reduce unnecessary ovarian damage. This is not clearly coming through in the manuscript especially if read by general audience of BMJ Open. In the current version, the added advantage of using intra-operative scanning vs simple pre-op scanning is not very clear.</p>
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	<p>16- PPI: well done for doing this! I would love to get more details of this meeting (date, venue, how were PPI recruited, etc..) to give this section a more formal presentation.</p> <p>This will be a fantastic study and I look forward to reading your findings.</p> <p>Regards</p> <p>Dr.Bassel H.Al Wattar MD PGD MRCOG PhD NIHR Clinical Research Associate Sub-specialty Trainee in Reproductive Medicine University College London Hospitals https://orcid.org/0000-0001-8287-9271</p>
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REVIEWER	Vignali, Michele Università degli Studi di Milano Facoltà di Medicina e Chirurgia, Department of Biomedical Sciences for Health
REVIEW RETURNED	05-Jan-2022

GENERAL COMMENTS	Thank you for the opportunity to review this manuscript. The topic is very interesting. Results could help to find a new way to reduce ovarian reserve impairment associated to ovarian cystectomy.
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REVIEWER	Ferrero, Simone Universita degli Studi di Genova
REVIEW RETURNED	10-Feb-2022

GENERAL COMMENTS	<p>The authors should clarify how intraoperative ultrasound may improve ovarian tissue preservation. How does the scan help? How is surgery performed if the saline solution is added to the pelvis? Is the excision of the cyst performed under saline solution?</p> <p>The study should clarify if the intraoperative scan improves the operative time.</p> <p>Does the intraoperative scan affect the intraoperative hemoglobin drop?</p>
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REVIEWER	Guerriero, Stefano University of Cagliari
REVIEW RETURNED	24-Feb-2022

GENERAL COMMENTS	<p>I read with interest this manuscript about a protocol to evaluate the role of Intra-operative Ultrasound Guided Laparoscopic Ovarian Cystectomy (UGLOC) as method to improve the fertility preservation in the surgical management of benign ovarian cysts. Intraoperative ultrasound is already commonly used in surgery for the liver for hepatic metastatic disease and hepatocellular carcinoma, in the pancreas for neuroendocrine tumors, in the kidney for renal cell carcinoma [1] and in the fertility-sparing surgery for borderline ovarian tumor [2]. I suggest to add in the Introduction the uses of intraoperative ultrasound already present in literature.</p> <p>In addition, even if I find this protocol interesting, some criticisms are present. In particular:</p> <ul style="list-style-type: none"> - It is not clear how the sample size was calculated. It is based on an absolute value and not on the rates of decline of AMH and follicular count. Randomized controlled trial is a clinical trial performed to reduce bias when proposing a new treatment. The number of patients (subjects or groups of subjects) assigned to
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	<p>control and treatment groups, affects a randomized controlled trial's reliability. If the effect of the treatment is small (and in the present study it seems small), the number of patients may be insufficient for rejecting the null hypothesis in the respective statistical test. The authors should add a clear calculation of the sample size in the revised manuscript based on the rates of decline of AMH and follicular count (in case of bilateral cyst).</p> <ul style="list-style-type: none"> - The inclusion of only bilateral cysts introduces a second critical bias. It would be useful to add an explanation for this choice. It is known that the presence of bilateral cysts in the case of endometriosis correlates with a greater decline in AMH [3]. In addition, regarding the 5 cases included in pilot study the characteristics of cysts are not described. Also, the follicular counts are not considered. I think it is useful to describe in detail the characteristics of the patients included. - In addition, the type of cyst could lead to a bias [4,5]. For example, in case of endometriotic cysts part of the ovarian damage may be due to the presence of the endometrioma itself [6]. This important bias should be reported in the Discussion. Perhaps a further subdivision between endometriomas and other types of benign cysts could be considered. - To conclude, other characteristics that could lead to biases, if the study group and control group are not correctly divided, are patient ethnicity, race and age [7]. These possible biases should be reported in the Discussion. <ol style="list-style-type: none"> 1. Lubner MG, Mankowski Gettle L, Kim DH, Ziemlewicz TJ, Dahiya N, Pickhardt P. Diagnostic and procedural intraoperative ultrasound: technique, tips and tricks for optimizing results. <i>Br J Radiol.</i> 2021 May 1;94(1121):20201406 2. Mascilini F, Quagliozi L, Bolomini G, Scambia G, Testa AC, Fagotti A. Intraoperative ultrasound through laparoscopic probe in fertility-sparing surgery for borderline ovarian tumor recurrence. <i>Ultrasound Obstet Gynecol.</i> 2019 Aug;54(2):280-282. doi: 10.1002/uog.20138. PMID: 30288807. 3. Hirokawa W, Iwase A, Goto M, Takikawa S, Nagatomo Y, Nakahara T, Bayasula B, Nakamura T, Manabe S, Kikkawa F. The post-operative decline in serum anti-Mullerian hormone correlates with the bilaterality and severity of endometriosis. <i>Hum Reprod.</i> 2011 Apr;26(4):904-10 4. Perlman S, Kjer JJ. Ovarian damage due to cyst removal: a comparison of endometriomas and dermoid cysts. <i>Acta Obstet Gynecol Scand.</i> 2016 Mar;95(3):285-90 5. Lind T, Hammarström M, Lampic C, Rodriguez-Wallberg K. Anti-Müllerian hormone reduction after ovarian cyst surgery is dependent on the histological cyst type and preoperative anti-Müllerian hormone levels. <i>Acta Obstet Gynecol Scand.</i> 2015 Feb;94(2):183-90 6. Kitajima M, Defrère S, Dolmans MM, Colette S, Squifflet J, Van Langendonck A, Donnez J. Endometriomas as a possible cause of reduced ovarian reserve in women with endometriosis. <i>Fertil Steril.</i> 2011 Sep;96(3):685-91 7. Kotlyar AM, Seifer DB. Ethnicity/Race and Age-Specific Variations of Serum AMH in Women-A Review. <i>Front Endocrinol (Lausanne).</i> 2021 Feb 9;11:593216
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VERSION 1 – AUTHOR RESPONSE

Reviewer one:

1) 'From a philosophical point, I think it is important to specify if you are really doing a "fertility preservation surgical technique" or you are proposing a "diagnostic adjunct" to an established surgical technique. You are not really changing the surgical course, but rather, changing the tools supporting surgical decision making.'

Although we appreciate that the use of intraoperative ultrasound could be considered an adjunct to an established surgical method for the management of benign ovarian cysts, we would consider intra-operative ultrasound guided cystectomy a novel method of fertility preservation surgery. The use of ultrasound not only supports clinical decision making, but it also encourages adaptation of the surgical technique from the blind resection of ovarian tissue, to a more precise method, with a potential to improve the rate of complete resection of pathology. Thus, we feel the addition of intra operative ultrasound does change the surgical course, therefore the preferred term should be referred to as a method of fertility preservation surgery.

2) 'I think it is important you implement and describe a standardised protocol for intra-operative scanning. Cannot have saline injected in some patients and not injected in others as this will increase performance bias especially since your sample size is small.'

We thank the reviewer for this comment. We acknowledge that the initial description of the procedure implied infiltration of normal saline into the pelvis required during intra-operative ultrasound would only be considered in a few women. We have now clarified that this is in fact standard protocol for all women allocated to this group. (Line 262-272; Page 10).

3) 'I tried to replicate your sample size calculation, but I failed (Table 2 is bit missed up in the PDF so could not follow the numbers). To have a high degree of certitude evaluating effectiveness (I.E if USS works in real world), I find having 16 patients in each arm too small to cover the variation in baseline characteristics that could affect your outcomes (high bmi, low ovarian reserve at baseline, pregnancy, adhesions, etc..). I appreciate doing surgical RCTs is tough. Has this calculation been adjusted for variation in patient characteristics? Maybe worth seeking an opinion from a statistician with trial expertise.'

We thank the reviewer for this constructive feedback. We have since sought the advice of the assigned Statistician for the Trial. We have provided an explanation of the power calculation, which we believe can be replicated when using the TrialSize package for a 2 sample mean for superiority or non-inferiority trials with R Statistical Programming version 4.2.0. The calculation has taken data from a Pilot study into consideration. Please find a detailed explanation (Line 345-381; Page 13).

4) 'I am a bit sceptical of your inclusion criteria for the following reasons:

a) 'The age range presented is too wide (up to 50 years old!). Invariably some of these 32 women will be peri-menopausal at/around 50 (an exclusion criterion set by you later. Also, women aged 45+ will have very little variations in their AMH purely due to poor ovarian reserve and it will skew your findings towards the null.'

We thank the reviewer for this important feedback and agree that women aged between 45-50 years old should be excluded from the study, as the majority will have a starting poor ovarian reserve. From our literature review, reproductive age is considered up to <45 years old, therefore this will now be the upper limit for the age cut off in this study.

b) 'Finally, why include pregnant women! GA has an effect on the baby and usually would not operate on a benign cyst in pregnancy unless it is complicated (e.g. Torsion).'

We agree with this comment and have therefore added pregnant women to the exclusion criteria.

c) 'The last item says (bilateral cysts): does this mean you won't include those with unilateral cyst? Why so?

We apologise for this error and thank the reviewer for drawing this to our attention. Bilateral ovarian cysts will not be included in the study, as this could be considered a confounding variable when comparing ovarian reserve in women who have only undergone unilateral cystectomy. We have since moved 'bilateral cysts' into the exclusion criteria.

5) 'The Abstract should not contain references'

The references have now been removed from the abstract accordingly.

6) 'The introduction is far too long. It reads like a chapter in a dissertation. For benefit of BMJ Open I think it can be shortened and made more concise.'

We have since reviewed the introduction and tried to ensure this is concisely written.

7) 'Ln115: I disagree that (fertility preservation) is a sub-speciality on its own. It's an area or topic of expertise'

Thank you for the suggestion. We have amended the sentence as follows: (Line 85-88; Page 4).

'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.' (Line 85-88; Page 4).

8) 'Ln131: I think you mean fertility rather than infertility?'

Many thanks for pointing this out. The word 'infertility' has been changed accordingly to 'fertility'.

9) 'Why an increasing demand for the implementation of fertility sparing surgical techniques for women with benign pathology is also prevalent): increasing and prevalent is kind of a double positive in one sentence.'

The sentence has been changed as follows:

'Considering the lifetime risk of women undergoing surgery for the presence of benign ovarian pathology is 5-10%, it is perhaps understandable why there is an increasing demand of fertility sparing surgical techniques for women with benign pathology.' (Line 110-114; Page 4).

10) 'There is so much confusion in the use of the terms: participants, patients, women, females throughout the manuscript: please standardise'

We agree with this comment and have therefore standardised the term, such that any woman participating in the trial is now referred to as a 'participant.' Prior to specific details of the study design and methods, they are referred to as 'woman'.

11) 'Similarly there is so much confusion with using (Fertility Sparing) and (fertility preservation) interchangeably, please standardise'

Apologies for this inconsistency throughout the protocol. We have now standardised the term to 'fertility preservation' throughout the manuscript.

12) 'Therefore, intraoperative ultrasound has the potential to improve surgical accuracy, reduce complications and improve patient safety.): making this argument in the introduction feels like you already make the conclusion before doing the study. I am not sure it increases safety, arguably introducing an additional (experimental) element to surgery in untrained hands can increase risk of complications (e.g. bowel injury).'

We thank the reviewer and agree with this comment. Particularly, as the statement was not supported by evidence or a reference. We have therefore deleted this, which has helped to shorten the introduction.

13) 'The aim/hypothesis/objectives are very confusing and need revision.'

a)'Ln172: To evaluate the effect of what?'

We have reworded the aim as follows: (Line 145-148; Page 6)

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

b)' Ln176: (intra-operative ultrasound as a method of fertility preservation surgery): first time your present ultrasound as a fertility preservation surgery "method". Not sure if actually ultrasound can be defined on its own as a surgical method.'

This query has been addressed already in comment one.

c) 'Please be mindful of the use of efficacy vs effectiveness'

We have amended the primary objective as follows: (Line 151-153; Page 6).

'To compare the rate of serum decline of AMH and AFC number at 3 and 6 months post operatively in women who have undergone intraoperative ultrasound guided ovarian cystectomy and compare to the control group.'

14) 'How do you plan to define (peri-menopausal women)?'

We have now included in the prose a few sentences on how we will define if women should be considered either peri- or post-menopausal status (Line 218-220; Page 8).

15) 'Please specify that the randomisation envelopes are opaque'

We have now specified that the envelopes will be opaque (Line 234; Page 9).

16) Is chronologically the correct word? I think they are in ascending order, but not sure of the element of time here. I'll leave this to the editors to judge.

We have changed the word 'chronological' to 'ascending order' instead. (Line 235; Page 9).

17) 'Ln270: I think the length of hospital stay must be standardised or it will affect the accuracy of this secondary outcome.'

The length of hospital stay cannot be standardised, as it will depend on how the patient recovers from the surgery and whether there are post-operative outcomes. This secondary outcome is to identify whether the use of intra operative ultrasound increases the duration of hospital stay or not. To standardize the protocol we have advised that all women undergoing surgery will have a minimum of 1 overnight stay in hospital. (Line 250-252; Page 10).

18) 'Ln276: Need Reference for BSGE'

This reference has now been added accordingly. (Reference No. 7).

19) 'I think it is better to describe how the concomitant use of ultrasound intraoperatively will improve tissue handling and reduce unnecessary ovarian damage. This is not clearly coming through in the manuscript especially if read by general audience of BMJ Open. In the current version, the added advantage of using intra-operative scanning vs simple pre-op scanning is not very clear.'

We thank the reviewer for this feedback. The advantages of intra-operative scanning have been described in the Introduction (Lines 127-142; Page 5). Such advantages include improvement of the operative field, reduced intraoperative complications through less injury to surrounding tissues and organs and increased incidence of lesions too small for the naked eye. There is also an explanation of how the scan allows the surgeon to demarcate between pathology and healthy ovarian tissue. There is no evidence or description to suggest that the use of intra operative ultrasound directly affects the handling of ovarian tissue itself, as essentially the same instruments are used to resect ovarian tissue, which is why no reference to this has been made.

20) ' PPI: well done for doing this! I would love to get more details of this meeting (date, venue, how were PPI recruited, etc..) to give this section a more formal presentation.'

The PPI meeting was carried out following a suggestion from the IRAS ethics review panel. Following advice from Imperial College London ethics committee, 10 women were selected from outpatient Gynaecology clinics at random to participate in reviewing the patient resources (including a patient information leaflet (PIS) and consent form as part of the proposed study). On agreement to review the resources, a copy of the PIS and consent form was given to take home and read. After a week, an online focus group meeting was scheduled, where all women attended the session to provide feedback on the resources. Some of the suggestions made have since been incorporated into the patient resources.

Reviewer 2

Many thanks for reviewer two's comments and feedback.

Reviewer 3

1) 'The authors should clarify how intraoperative ultrasound may improve ovarian tissue preservation. How does the scan help?'

We have included a subheading within the Introduction called 'Intra operative ultrasound' which discusses the advantages of intra operative ultrasound, such as improvement of the operative field, reduced intraoperative complications through less injury to surrounding tissues and organs and increased incidence of lesions too small for the naked eye. There is also an explanation of how the scan allows the surgeon to demarcate between pathology and healthy ovarian tissue. (Lines 122-142; Page 5).

2) How is surgery performed if the saline solution is added to the pelvis? Is the excision of the cyst performed under saline solution?'

We thank the reviewer for this feedback as we appreciate the initial description of the procedure was vague. We have now provided a clearer and thorough explanation of the procedure on (Page 10; Line 263-272).

3) 'The study should clarify if the intraoperative scan improves the operative time.'

We could not find evidence to suggest that the use of intra operative ultrasound increases the duration of surgery. For this reason, we will assess the duration of surgery (minutes) as one of the secondary outcomes of the study.

4) 'Does the intraoperative scan affect the intraoperative haemoglobin drop?'

There is no evidence to suggest that the use of an intra operative scan would affect the Haemoglobin concentration. We have not considered this as a secondary outcome because it would be difficult to attribute any drop of haemoglobin to the use of the ultrasound specifically and account for other confounding variables. For example, if the surgery is complicated and resection of the cyst causes bleeding from the base of the ovarian tissue, this may not be a direct consequence from the intra operative scan performed.

Reviewer: 4

1) 'Intraoperative ultrasound is already commonly used in surgery for the liver for hepatic metastatic disease and hepatocellular carcinoma, in the pancreas for neuroendocrine tumors, in the kidney for renal cell carcinoma (1) and in the fertility-sparing surgery for borderline ovarian tumor (1). I suggest to add in the Introduction the uses of intraoperative ultrasound already present in literature.'

We thank the reviewer for this feedback and the list of references which are a useful addition to the manuscript. We have now included the use of intra operative ultrasound observed in other specialities. (Line 123-126; Page 5).

2) It is not clear how the sample size was calculated. It is based on an absolute value and not on the rates of decline of AMH and follicular count. Randomized controlled trial is a clinical trial performed to reduce bias when proposing a new treatment. The number of patients (subjects or groups of subjects) assigned to control and treatment groups, affects a randomized controlled trial's reliability. If the effect of the treatment is small (and in the present study it seems small), the number of patients may be insufficient for rejecting the null hypothesis in the respective statistical test. The authors should add a clear calculation of the sample size in the revised manuscript based on the rates of decline of AMH and follicular count (in case of bilateral cyst).

We thank the reviewer for this constructive feedback. We have since sought the advice of the assigned Statistician for the Trial. We have provided an explanation of the power calculation, which we believe should be easy to replicate when using the TrialSize package for a 2 sample mean for superiority or non-inferiority trials with R Statistical Programming version 4.2.0. The calculation has taken data from a Pilot study into consideration. Please find a detailed explanation on (Line 345-381; Page 13)

3) 'The inclusion of only bilateral cysts introduces a second critical bias. It would be useful to add an explanation for this choice. It is known that the presence of bilateral cysts in the case of endometriosis correlates with a greater decline in AMH (1).'

We apologise as the inclusion of bilateral cysts was an error, as also pointed out by reviewer one. We have now moved bilateral ovarian cysts to the exclusion criteria, for the reasons the reviewer states.

4) In addition, regarding the 5 cases included in pilot study the characteristics of cysts are not described. Also, the follicular counts are not considered. I think it is useful to describe in detail the characteristics of the patients included.

The 5 women included in the Pilot study underwent ultrasound guided ovarian cystectomy for borderline ovarian tumours. This information has been provided on (Line 357; Page 13). Having reviewed a number of research protocols published by BMJ Open which have derived power calculations from Pilot data, we observed that many did not provide comprehensive information regarding the participant characteristics. Whilst we appreciate this is important information, we found that when including such data, it over complicated the description of the power calculation, making it harder to follow. As such, we have followed the template of previous publications and provided a very concise description of the power calculation, which we feel is replicable and easy to follow.

5) 'In addition, the type of cyst could lead to a bias [4,5]. For example, in case of endometriotic cysts part of the ovarian damage may be due to the presence of the endometrioma itself [6]. This important bias should be reported in the Discussion. Perhaps a further subdivision between endometriomas and other types of benign cysts could be considered.'

We agree that the type of cyst will introduce bias to the study, particularly in the case of endometriomas. We have therefore decided to exclude cysts of this pathology from the study. We did consider whether to include them and perform a subgroup analysis as excellently suggested by the reviewer. However, we felt it would complicate the primary and secondary aims of the study, and in fact the use of intra operative ultrasound for the management of endometriomas could be a separate study in itself. We have referred to this under a new sub heading entitled "potential bias to the study.' (Line 403-409; Page 15). We thank the reviewer for the suggested references also.

6) 'To conclude, other characteristics that could lead to biases, if the study group and control group are not correctly divided, are patient ethnicity, race and age [7]. These possible biases should be reported in the Discussion.'

We have discussed the potential bias of participant characteristics under the heading potential bias to the study' and included the reference suggested. (Line 411-415; Page 16).

The authors have amended the manuscript as per all reviewers' feedback, which we feel has strengthened the research design and structure of the manuscript. We hope you find the revised version and answers to the reviewers comments satisfactory for you to accept this protocol in your

journal. Please do not hesitate to contact me if we can be of any further assistance in the revision of this document, which will hopefully lead to its final acceptance.

VERSION 2 – REVIEW

REVIEWER	Al Wattar, Bassel Birmingham Women's Hospital, Obstetrics and Gynaecology
REVIEW RETURNED	22-Jun-2022

GENERAL COMMENTS	The authors have addressed all raised comments satisfactorily. I wish them all the success in conducting this important study. Dr.Bassel H.Al Wattar MD PGD MRCOG PhD NIHR Clinical Research Associate Sub-specialty Trainee in Reproductive Medicine University College London Hospitals https://orcid.org/0000-0001-8287-9271
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REVIEWER	Guerriero, Stefano University of Cagliari
REVIEW RETURNED	16-Jun-2022

GENERAL COMMENTS	All the suggestions have been included in the revised version.
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