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- Comments from the reviewers and editors (email to author requesting revisions)
- Response from the author (cover letter submitted with revised manuscript)*

*The corresponding author has opted to make this information publicly available.

Personal or nonessential information may be redacted at the editor’s discretion.

Questions about these materials may be directed to the Obstetrics & Gynecology editorial office: obgyn@greenjournal.org.
RE: Manuscript Number ONG-22-934

A Longitudinal Assessment of Ovarian Reserve following Myomectomy

Dear Dr. Aharon:

Thank you for sending us your work for consideration for publication in Obstetrics & Gynecology. Your manuscript has been reviewed by the Editorial Board and by special expert referees. The Editors would like to invite you to submit a revised version for further consideration.

If you wish to revise your manuscript, please read the following comments submitted by the reviewers and Editors. Each point raised requires a response, by either revising your manuscript or making a clear argument as to why no revision is needed in the cover letter.

To facilitate our review, we prefer that the cover letter you submit with your revised manuscript include each reviewer and Editor comment below, followed by your response. That is, a point-by-point response is required to each of the EDITOR COMMENTS (if applicable), REVIEWER COMMENTS, STATISTICAL EDITOR COMMENTS (if applicable), and EDITORIAL OFFICE COMMENTS below. Your manuscript will be returned to you if a point-by-point response to each of these sections is not included.

The revised manuscript should indicate the position of all changes made. Please use the "track changes" feature in your document (do not use strikethrough or underline formatting).

Your submission will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Jul 08, 2022, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1:

Summary of the study:
This is a prospective cohort study of patients with fibroids undergoing open myomectomy (OM) or minimal invasive myomectomy (MIM) to evaluate the anti-Mullerian hormone (AMH) levels at preoperative and different postoperative time points. The study reported significant decrease of AMH from baseline at 2 weeks among overall participants and those underwent OM compared with MIM. The AMH levels at 3 and 6 months after surgery did not differ from baseline. The study concluded that myomectomy, particularly OM, is associated with a transient decline in AMH levels, however, is not associated with a long-term impact on ovarian reserve.

Abstract
- Line 16: As the AMH level is associated with age, it may be worthwhile to describe age of overall population in the study.
- Line 17, 20, 22: Please consider adding the unit of AMH levels.

Introduction
- The background and rational for the study are clearly written.

Methods
- Line: 65: One limitation that should be pointed out is that the assignment for OM and MIM was determined based on size, location, and number of fibroids, which could affect the AMH levels. Thus, the results of different AMH level between OM and MIM could be due to confounding by indication for treatment.
- Line 71: Did patients receive any postoperative medications that could affect the AMH levels?
- Line 102: Is AMH level normally distributed? It is confusing as the author mentioned to use Wilcoxon Signed Rank for...
paired analysis, however, reported results as means instead of as medians. If AMH is not normally distributed, was it transformed in the regression model?

Results
-Line 116: Although the study recruited 111 patients, however, the patients included in the analysis was 87 who presented at one or more follow-up time points. Thus, the author should consider presenting demographics among 87 patients in the analysis after excluding 24 patient who withdrew from the study instead of 111 patients.

-Table 1: Gravidity and parity may better be reported as median and IQR then compared with Wilcoxon Rank Sum test. In addition to fibroid weight, did you collect the information on location and number of fibroids. If the data is available, it may be worthwhile to report.

-Table 2: Should consider adding sample size at each time point of comparison. Please add the unit of AMH. As the table presented means and mean differences, should we assume that the AMH is normally distributed and compared to baseline with paired t-test instead of Wilcoxon Signed Rank for paired analysis as mentioned in the method section? Please clarify on the methods used for the analysis.

-Table 3: Should consider adding sample size at each time point of comparison.

-Figure 1: The author may consider adding the information on 87 patients used in the analysis who presented at one or more follow-up time points. Among 87 patients, 62, 47, 41 contributed to follow-up visit at 2 week, 3 months, and 6 months, respectively.

-Paragraph from Line 141 and Figure 2: Were the comparisons of mean change between different time points done by paired test as to compare AMH levels from the same group of population.

Discussion
-The author pointed out the important points that different AMH levels could be due to the case selection and surgical technique using tourniquet.

-There was loss to follow up, please explain the possibility of limited sample size contributing to the non-significantly different of AMH levels.

-Line 190: The authors described the trend towards an increased AMH level from baseline to 3 and 6 months. Please discuss the possibility of bias from study drop out as mean AMH at different time points were based on different sample size.

-The author may consider a spaghetti plot to depict clearer trend in the change on AMH level across different time points.

Reviewer #2:

Dear Authors,

Congratulations on a very interesting prospective study to assess whether open and minimally invasive myomectomy is associated with changes in post-operative ovarian reserve.

Comments:
1. Overall, the manuscript is well-written with few grammatical errors.

2. In the results section of the abstract (line 16), stating your findings for changes in AMH levels among the 111 study patients is deceiving as there is a high attrition rate due to subject lost to follow-up as reported later in your manuscript.

3. Line 34 - recommend mentioning different types of medical suppressive therapies rather than a range.

4. Line 35 - Procedures that were left off include radiofrequency ablation procedures in addition to UAE, myomectomy, and hysterectomy.

5. Lines 40-42 - Should mention what these "similar mechanisms" are rather than leave it vague.

6. Lines 47-52 is well-summarized and important as the overall impact of this study.

7. Line 56 - are tourniquets always used in open myomectomies?

8. Lines 81-82 - it is not clearly mentioned if the utero-ovarian ligaments were clamped as the tourniquet was applied at the base of the uterus around the uterine and "ovarian vessels." Please clarify.
9. Lines 93-99, 114-115 - An apriori power analysis was calculated which revealed a sample size of 43 subjects to detect a 15% difference in mean AMH level with 80% power and an alpha of 0.05. What is this based on? In the results section of the manuscript, only 41 patients presented for 6 month follow up and therefore, can the conclusion that there was no difference in AMH level at this time period be made?

10. Lines 109-115 - how many open vs minimally invasive myomectomy should be mentioned in the text of the manuscript rather in just Figure 1.

11. Line 166 - how small were the number of subjects in these other studies?

12. Lines 168-171 - were there any patients that required blood transfusion? This should be mentioned.

13. Lines 190-195 - Does impact of blood flow affect ovarian function? What evidence is provide by this statement or is it just speculation?

14. Lines 200-204 - Attrition bias is understated as a limitation in the manuscript. Can the conclusion be made at 6 months post-op with just 41 patients in the analysis?

15. Table 2 - should include the number of patients in each group

16. Figure 1 - Perhaps the discrepancy with the difference in each of the OM (40) and MIM (22) group at the 2 week postop followup accounts for the stated results?

Reviewer #3: The current manuscript presents data from a prospective cohort study to evaluate the impact of open and minimally invasive myomectomy on ovarian reserve post-operatively as measured by AMH testing. The cases were performed by a single surgeon with AMH testing pre-operatively and post-operatively at 2 weeks, 3 months and 6 months. All abdominal cases were performed with a tourniquet while laparoscopic cases utilized vasopressin.

1. Introduction, lines 40-42: Briefly state what the theorized mechanism is to decrease ovarian reserve.

2. Methods, lines 75-78: Were the samples all analyzed at the same time? If not, were the study authors and surgeons aware of the pre-operative AMH?

3. Methods, line 82: Was vasopressin used at all during open myomectomy? Were any type of clamps or tourniquet used laparoscopically?

4. Methods, line 89: Were any patients treated with oral contraceptives before or after surgery? It is known that OCPs can decrease the AMH level transiently.

5. Results: It seems that when looking at the change in AMH, the mean for each group at each timeline was compared. It would be helpful to additionally evaluate the mean change in AMH per patient at each time point and also to present that with a linear graph.

6. Discussion: The manuscript would benefit from additional discussion of AMH itself. It is felt to be very stable over time. What pathophysiologically is thought to cause this short-term decline that is able to be recovered? What other things have been shown to cause a temporary decrease in AMH?

STATISTICAL EDITOR COMMENTS:

Fig 1: The loss of sample size from the initial recruitment to 2 wk, 3 mo and 6 mo follow-up makes the findings potentially biased, since < 40% of the original cohorts were included.

Table 1: Gravidity and parity can only have integer values. Should format as median(range) or as categories and test with appropriate stats test. The AMH levels, EBL and fibroid wgts are formatted as mean ± SD (I assume). Need to state in Table or legend the format for the variables. Also, assuming those are means and SD, the relationship between their respective means and SDs implies skewed distributions. If the distributions were not normal, then need to either use a non-parametric test or transform the variables so that the distributions conform to normal, before applying a stats test that assumes normal distribution of the variables. Also, the SD for "All patients" fibroid wgt is too small, i.e., inconsistent with the SDs for its component subsets.
Table 1 (continued): The groups differed at baseline in BMI etc. Why did the Authors not adjust or match to convince the reader that the groups were comparable, other than surgical approach? Also, given the loss to follow-up, need to show the baseline characteristics of the cohorts at 2 weeks, 3 months and 6 months. Need to convince the reader that the pairwise analyses of those groups are representative of and generalizable to the initial group, summarized in Table 1.

Table 2: Since these are paired differences, then need to cite in appropriate column how many pairs were included in each column of mean differences.

General: While this analysis is appropriate for a descriptive series (subject to the limitation of loss of follow-up) or as comparing the two surgical approaches, it seems to lack a control group. That is, is the decrease at two weeks unique to open myomectomy, or would it also occur with other types of open abdominal surgery besides myomectomy?

EDITORIAL OFFICE COMMENTS:

1. If your article is accepted, the journal will publish a copy of this revision letter and your point-by-point responses as supplemental digital content to the published article online. You may opt out by writing separately to the Editorial Office at em@greenjournal.org, and only the revision letter will be posted.

2. When you submit your revised manuscript, please make the following edits to ensure your submission contains the required information that was previously omitted for the initial double-blind peer review:
   * Funding information (ie, grant numbers or industry support statements) should be disclosed on the title page and at the end of the abstract. For industry-sponsored studies, describe on the title page how the funder was or was not involved in the study.
   * Include clinical trial registration numbers, PROSPERO registration numbers, or URLs at the end of the abstract (if applicable).
   * Name the IRB or Ethics Committee institution in the Methods section (if applicable).
   * Add any information about the specific location of the study (ie, city, state, or country), if necessary for context.

3. Obstetrics & Gynecology's Copyright Transfer Agreement (CTA) must be completed by all authors. When you uploaded your manuscript, each coauthor received an email with the subject, "Please verify your authorship for a submission to Obstetrics & Gynecology." Please ask your coauthor(s) to complete this form, and confirm the disclosures listed in their CTA are included on the manuscript's title page. If they did not receive the email, they should check their spam/junk folder. Requests to resend the CTA may be sent to em@greenjournal.org.

4. ACOG uses person-first language. Please review your submission to make sure to center the person before anything else. Examples include: "People with disabilities" or "women with disabilities" instead of "disabled people" or "disabled women"; "patients with HIV" or "women with HIV" instead of "HIV-positive patients" or "HIV-positive women"; and "people who are blind" or "women who are blind" instead of "blind people" or "blind women."

5. The journal follows ACOG's Statement of Policy on Inclusive Language (https://www.acog.org/clinical-information/policy-and-position-statements/statements-of-policy/2022/inclusive-language). When possible, please avoid using gendered descriptors in your manuscript. Instead of "women" and "females," consider using the following: "individuals," "patients;" "participants;" "people" (not "persons"); "women and transgender men;" "women and gender-expansive patients;" or "women and all those seeking gynecologic care."

6. Obstetrics & Gynecology follows the Good Publication Practice (GPP3)* guideline for manuscripts that report results that are supported or sponsored by pharmaceutical, medical device, diagnostics and biotechnology companies. The GPP3 is designed to help individuals and organization maintain ethical and transparent publication practices.

   (1) Adherence to the GPP3 guideline should be noted in the cover letter.

   (2) For publication purposes, the portions of particular importance to industry-sponsored research are below. In your cover letter, please indicate whether the following statements are true or false, and provide an explanation if necessary:

   (2a) All authors had access to relevant aggregated study data and other information (for example, the study protocol) required to understand and report research findings.

   (2b) All authors take responsibility for the way in which research findings are presented and published, were fully involved at all stages of publication and presentation development and are willing to take public responsibility for all aspects of the work.

   (2c) The author list accurately reflects all substantial intellectual contributions to the research, data analyses, and publication or presentation development. Relevant contributions from persons who did not qualify as authors are disclosed in the acknowledgments.

   (2d) The role of the sponsor in the design, execution, analysis, reporting, and funding (if applicable) of the research has been fully disclosed in all publications and presentations of the findings. Any involvement by persons or organizations with
an interest (financial or nonfinancial) in the findings has also been disclosed.

(2e) All authors have disclosed any relationships or potential competing interests relating to the research and its publication or presentation.

(3) The end of the abstract should contain the heading, "Funding Source," and should provide an abbreviated listing of the funder(s).

(4) The title page should describe how the funder was or was not involved in the manuscript.

7. Please add whether you received IRB or Ethics Committee approval or exemption to your Methods. Include the name of the IRB or Ethics Committee. If you received an exemption, explain why in this section.

8. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

9. Make sure your manuscript meets the following word limit. The word limit includes the manuscript body text only (for example, the Introduction through the Discussion in Original Research manuscripts), and excludes the title page, précis, abstract, tables, boxes, and figure legends, reference list, and supplemental digital content. Figures are not included in the word count.

Original Research: 3,000 words

10. Specific rules govern the use of acknowledgments in the journal. Please review the following guidelines and edit your title page as needed:

* All financial support of the study must be acknowledged.
* Any and all manuscript preparation assistance, including but not limited to topic development, data collection, analysis, writing, or editorial assistance, must be disclosed in the acknowledgments. Such acknowledgments must identify the entities that provided and paid for this assistance, whether directly or indirectly.
* All persons who contributed to the work reported in the manuscript, but not sufficiently to be authors, must be acknowledged. Written permission must be obtained from all individuals named in the acknowledgments, as readers may infer their endorsement of the data and conclusions. Please note that your response in the journal's electronic author form verifies that permission has been obtained from all named persons.
* If all or part of the paper was presented at the Annual Clinical and Scientific Meeting of the American College of Obstetricians and Gynecologists or at any other organizational meeting, that presentation should be noted (include the exact dates and location of the meeting or indicate whether the meeting was held virtually).
* If your manuscript was uploaded to a preprint server prior to submitting your manuscript to Obstetrics & Gynecology, add the following statement to your title page: "Before submission to Obstetrics & Gynecology, this article was posted to a preprint server at: [URL]."
* Do not use only authors' initials in the acknowledgement or Financial Disclosure; spell out their names the way they appear in the byline.

11. Provide a short title of no more than 45 characters, including spaces, for use as a running foot. Do not start the running title with an abbreviation.

12. Be sure that each statement and any data in the abstract are also stated in the body of your manuscript, tables, or figures. Statements and data that appear in the abstract must also appear in the body text for consistency. Make sure there are no inconsistencies between the abstract and the manuscript, and that the abstract has a clear conclusion statement based on the results found in the manuscript.

In addition, the abstract length should follow journal guidelines. Please provide a word count.

Original Research: 300 words

13. Only standard abbreviations and acronyms are allowed. A selected list is available online at http://edmgr.ovid.com/ong/accounts/abbreviations.pdf. Abbreviations and acronyms cannot be used in the title or précis. Abbreviations and acronyms must be spelled out the first time they are used in the abstract and again in the body of the manuscript.

14. ACOG avoids using "provider." Please replace "provider" throughout your paper with either a specific term that defines the group to which are referring (for example, "physicians," "nurses," etc.), or use "health care professional" if a specific term is not applicable.

15. In your abstract, manuscript Results sections, and tables, the preferred citation should be in terms of an effect size,
such as odds ratio or relative risk or the mean difference of a variable between two groups, expressed with appropriate confidence intervals. When such syntax is used, the P value has only secondary importance and often can be omitted or noted as footnotes in a Table format. Putting the results in the form of an effect size makes the result of the statistical test more clinically relevant and gives better context than citing P values alone.

Please standardize the presentation of your data throughout the manuscript submission. For P values, do not exceed three decimal places (for example, "P = .001").

Express all percentages to one decimal place (for example, 11.1\%). Do not use whole numbers for percentages.

16. Please review the journal’s Table Checklist to make sure that your tables conform to journal style. The Table Checklist is available at http://edmgr.ovid.com/ong/accounts/table_checklist.pdf.

17. Please review examples of our current reference style at https://edmgr.ovid.com/ong/accounts/ifa_suppl_refstyle.pdf. Include the digital object identifier (DOI) with any journal article references and an accessed date with website references.

Unpublished data, in-press items, personal communications, letters to the editor, theses, package inserts, submissions, meeting presentations, and abstracts may be included in the text but not in the formal reference list. Please cite them on the line in parentheses.

If you cite ACOG documents in your manuscript, be sure the references you are citing are still current and available. Check the Clinical Guidance page at https://www.acog.org/clinical (click on "Clinical Guidance" at the top). If the reference is still available on the site and isn't listed as "Withdrawn," it's still a current document. In most cases, if an ACOG document has been withdrawn, it should not be referenced in your manuscript.

Please make sure your references are numbered in order of appearance in the text.

18. Figures 1-2: Please upload as figure files on Editorial Manager.

19. Authors whose manuscripts have been accepted for publication have the option to pay an article processing charge and publish open access. With this choice, articles are made freely available online immediately upon publication. An information sheet is available at http://links.lww.com/LWW-ES/A48. The cost for publishing an article as open access can be found at https://wkauthorservices.editage.com/open-access/hybrid.html.

If your article is accepted, you will receive an email from the Editorial Office asking you to choose a publication route (traditional or open access). Please keep an eye out for that future email and be sure to respond to it promptly.

***

If you choose to revise your manuscript, please submit your revision through Editorial Manager at http://ong.editorialmanager.com. Your manuscript should be uploaded as a Microsoft Word document. Your revision's cover letter should include a point-by-point response to each of the received comments in this letter. Do not omit your responses to the EDITOR COMMENTS (if applicable), the REVIEWER COMMENTS, the STATISTICAL EDITOR COMMENTS (if applicable), or the EDITORIAL OFFICE COMMENTS.

If you submit a revision, we will assume that it has been developed in consultation with your coauthors and that each author has given approval to the final form of the revision.

Again, your manuscript will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Jul 08, 2022, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Jason D. Wright, MD
Editor-in-Chief

2020 IMPACT FACTOR: 7.661
2020 IMPACT FACTOR RANKING: 3rd out of 83 ob/gyn journals

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/ong/login.asp?a=r). Please contact the publication office if you have any questions.
Dear Dr. Wright and Editorial Staff,

Thank you for your consideration and thoughtful review of our manuscript entitled “A Longitudinal Assessment of Ovarian Reserve Following Myomectomy.” The revised manuscript with tracked changes and with changes accepted are attached. A point-by-point response to the reviewers’ and Editorial Office’s comments are included at the end of this letter. Line numbers refer to the manuscript revised with tracked changes.

This manuscript is being submitted solely to Obstetrics and Gynecology. It is not under consideration elsewhere and will not be submitted elsewhere unless a final negative decision is made by the Editors of Obstetrics and Gynecology.

Declaration of transparency: The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained. Signed by: Devora Aharon *The manuscript’s guarantor

Attestation of self-blinding: I, Devora Aharon, have reviewed and edited the submission to omit any identifying information. I hereby submit this self-blinded manuscript for consideration in Obstetrics & Gynecology.

IRB approval: This study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai. Written informed consent was obtained from all study participants.

Previous presentation: This study was previously presented as an oral presentation at the Annual Meeting of the American Society for Reproductive Medicine in Philadelphia, Pennsylvania, October 12-16, 2019.

Reporting guidelines: This manuscript complies with STROBE guidelines for observational cohort studies. Please see enclosed checklist.

Disclosure of potential conflicts of interest: Dr. Alan Copperman is an advisor or board member of Sema 4, Progyny, and Celmatix. Charles Ascher-Walsh is a consultant for Gynesonics and owns Expert Alternatives which produces Fibrova, a vitamin supplement for fibroids. The remaining authors did not disclose any potential conflicts of interest.

This manuscript adheres to the GPP3 guideline. Please see itemized responses below.

Sincerely,

Devora Aharon, M.D.
Department of Obstetrics, Gynecology, and Reproductive Sciences
Icahn School of Medicine at Mount Sinai
Reproductive Medicine Associates of New York
New York, N.Y. 10029, USA
RESPONSE TO REVIEWERS:

REVIEWER COMMENTS:

Reviewer #1:

Summary of the study:
This is a prospective cohort study of patients with fibroids undergoing open myomectomy (OM) or minimal invasive myomectomy (MIM) to evaluate the anti-Mullerian hormone (AMH) levels at preoperative and different postoperative time points. The study reported significant decrease of AMH from baseline at 2 weeks among overall participants and those underwent OM compared with MIM. The AMH levels at 3 and 6 months after surgery did not differ from baseline. The study concluded that myomectomy, particularly OM, is associated with a transient decline in AMH levels, however, is not associated with a long-term impact on ovarian reserve.

Abstract
-Line 16: As the AMH level is associated with age, it may be worthwhile to describe age of overall population in the study.

The mean age has been added in line 16: “The study included 111 patients (mean age 37.9±4.7).”

-Line 17, 20, 22: Please consider adding the unit of AMH levels.

Thank you, AMH units of ng/mL have been added.

Introduction
-The background and rational for the study are clearly written.

Thank you.

Methods
-Line: 65: One limitation that should be pointed out is that the assignment for OM and MIM was determined based on size, location, and number of fibroids, which could affect the AMH levels. Thus, the results of different AMH level between OM and MIM could be due to confounding by indication for treatment.

The following has been added to the discussion (lines 242-244): “It is possible that differences in indication for treatment could be related to post-operative AMH levels, as route of surgery was determined based on size, location, and number of fibroids, which differed among the OM and MIM groups and might potentially impact AMH levels.”

-Line 71: Did patients receive any postoperative medications that could affect the AMH levels?

Data on post-operative medications was limited, as patients typically returned to their primary Gyn for long-term management. This limitation has been added to the discussion in lines 239-240: “Additionally, data on medication such as oral contraceptives was not consistently available, as many patients returned to their primary gynecologist for long-term management.”
Is AMH level normally distributed? It is confusing as the author mentioned to use Wilcoxon Signed Rank for paired analysis, however, reported results as means instead of as medians. If AMH is not normally distributed, was it transformed in the regression model?

The AMH level at each time point was not normally distributed, therefore in Table 1, the median baseline AMH is reported and Wilcoxon Rank-Sum was used for analysis. Mean differences at each time point were approximately normally distributed. Upon consultation with an additional statistician, we have changed the reporting to use p values obtained through paired t tests. We also compared the change in AMH using Wilcoxon Signed Rank and obtained the same results. The methods, results, and tables have been edited accordingly (lines 114, 153-156, 166-171, Table 2).

**Results**

Although the study recruited 111 patients, however, the patients included in the analysis was 87 who presented at one or more follow-up time points. Thus, the author should consider presenting demographics among 87 patients in the analysis after excluding 24 patient who withdrew from the study instead of 111 patients.

We wanted to show the information on all patients who enrolled and contributed a baseline AMH level and baseline data, but we agree that it is informative to include baseline information for the patients who contributed follow-up data. Demographic data among the 87 patients who presented for follow up, as well as the groups who contributed follow-up data at each time point, are shown in Appendix 1. The following has been added to the results (lines 145-149): “Baseline demographics among the 87 patients who presented for any follow-up, and for the 62 patients who presented at 2 weeks, the 47 patients who presented at 3 months, and the 41 patients who presented at 6 months are shown in Appendix 1. In each of these subgroups, baseline AMH level was similar among the OM and MIM groups, and other baseline characteristics followed similar trends compared to the group as a whole.”

Gravidity and parity may better be reported as median and IQR then compared with Wilcoxon Rank Sum test.

Table 1 has been edited. Gravidity, parity, and other parametric variables are expressed as median (interquartile range), and are compared using Wilcoxon Rank Sum test.

In addition to fibroid weight, did you collect the information on location and number of fibroids. If the data is available, it may be worthwhile to report.

This data was not available in a standardized manner and therefore was not reported.

Should consider adding sample size at each time point of comparison. Please add the unit of AMH. As the table presented means and mean differences, should we assume that the AMH is normally distributed and compared to baseline with paired t-test instead of Wilcoxon Signed Rank for paired analysis as mentioned in the method section? Please clarify on the methods used for the analysis.

Sample size has been added at each time point and unit of AMH has been added to the title of the table. As mentioned above, mean differences at each time point were approximately normally distributed. We have changed the reporting to use p values obtained through paired t tests. We also compared the change in AMH using Wilcoxon Signed Rank and obtained the same results. The methods, results, and tables have been edited accordingly (lines 114, 153-156, 166-171, Table 2).

Should consider adding sample size at each time point of comparison.
Sample size at each time point has been added to Table 3.

-Figure 1: The author may consider adding the information on 87 patients used in the analysis who presented at one or more follow-up time points. Among 87 patients, 62, 47, 41 contributed to follow-up visit at 2 week, 3 months, and 6 months, respectively.

This information has been added in Figure 1.

-Paragraph from Line 141 and Figure 2: Were the comparisons of mean change between different time points done by paired test as to compare AMH levels from the same group of population.

Table 2 demonstrates the results of paired analysis of mean change in AMH level. Figure 2 demonstrates the overall mean in each group at each time point and does not represent a paired analysis. Patients contributed to different follow-up time points, therefore it would not be possible to plot paired means at all time points longitudinally in the same figure, and so we chose to represent the overall means. See figure of a spaghetti plot plotting each patient’s baseline and follow-up values below.

The following has been added in lines 171-173: “Figure 2 demonstrates mean serum AMH levels among all patients who presented at each time point among the group overall and the OM and MIM groups.”

Discussion
- The author pointed out the important points that different AMH levels could be due to the case selection and surgical technique using tourniquet.

Thank you.

- There was loss to follow up, please explain the possibility of limited sample size contributing to the non-significantly different of AMH levels.

See lines 230-232: “Limitations of the study include loss of patient participation at follow-up, which may introduce bias and leads to a decreased ability to detect a significant difference in outcome at 6 months.”

-Line 190: The authors described the trend towards an increased AMH level from baseline to 3 and 6 months. Please discuss the possibility of bias from study drop out as mean AMH at different time points were based on different sample size.

This observation was based on paired analysis, so increases observed were in comparison to those same patients’ baselines. Each time point had a different sample size but the comparison was performed within the group of 62, 47, and 41, respectively.

-The author may consider a spaghetti plot to depict clearer trend in the change on AMH level across different time points.

A spaghetti plot depicting each patient’s AMH level at each time point is shown below. Because patients contributed different follow-up time points, there are a fair number of skipped time points so we feel that this figure is not very demonstrative.
Reviewer #2:

Dear Authors,

Congratulations on a very interesting prospective study to assess whether open and minimally invasive myomectomy is associated with changes in post-operative ovarian reserve.

Comments:
1. Overall, the manuscript is well-written with few grammatical errors.

Thank you.

2. In the results section of the abstract (line 16), stating your findings for changes in AMH levels among the 111 study patients is deceiving as there is a high attrition rate due to subject lost to follow-up as reported later in your manuscript.

This point is well-taken, the number of patients who contributed follow-up has been added in line 17: “87 patients contributed follow-up data.”

3. Line 34 - recommend mentioning different types of medical suppressive therapies rather than a range.

Lines 39-40 have been edited as follows “Treatment options for fibroids include medical suppressive therapies such as oral contraceptive pills, progesterone receptor modulators, and leuprolide acetate.”

4. Line 35 - Procedures that were left off include radiofrequency ablation procedures in addition to UAE, myomectomy, and hysterectomy.

Radiofrequency ablation has been added in line 41.
5. **Lines 40-42** - Should mention what these "similar mechanisms" are rather than leave it vague.

Lines 46-51 have been edited as follows: “Other invasive and minimally-invasive treatments for fibroids including uterine artery embolization and hysterectomy may decrease ovarian reserve, and it is possible that myomectomy may impact ovarian reserve through similar mechanisms, such as interrupting collateral vasculature supplying the ovaries through cautery, sutures, or temporary occlusion. The use of a tourniquet to minimize blood loss during myomectomy transiently decreases blood supply to the ovaries and could potentially harm ovarian reserve.”

6. **Lines 47-52** is well-summarized and important as the overall impact of this study.

Thank you.

7. **Line 56** - are tourniquets always used in open myomectomies?

In our study, tourniquets were used in all open myomectomies and were not in used in minimally-invasive myomectomies.

8. **Lines 81-82** - it is not clearly mentioned if the utero-ovarian ligaments were clamped as the tourniquet was applied at the base of the uterus around the uterine and "ovarian vessels."

The utero-ovarian ligaments were not clamped, rather the vessels traversing the IP would be clamped. The following has been added in lines 88-90: “The uterus was exteriorized through the abdominal incision and the drain was tied at the base of the uterus around the uterine and ovarian vessels, incorporating the ovaries and fallopian tubes within the tourniquet.”

9. **Lines 93-99, 114-115** - An apriori power analysis was calculated which revealed a sample size of 43 subjects to detect a 15% difference in mean AMH level with 80% power and an alpha of 0.05. What is this based on? In the results section of the manuscript, only 41 patients presented for 6 month follow up and therefore, can the conclusion that there was no difference in AMH level at this time period be made?

We based on our effect on size on prior literature and our determination that 15% would be a clinically meaningful change. Yuan et. al. detected significant differences in AMH level of about 15% following hysterectomy, and Wang et. al. detected about a 20% decline in AMH following hysterectomy but did not detect a significant change following myomectomy with power to detect a difference of 30%. “Effect size was determined based prior studies” has been added in line 111.

Lines 236-239 have been modified to state: “Limitations of the study include loss of patient participation at follow-up, which may introduce bias and leads to a decreased ability to detect a significant difference in outcome at 6 months.”

10. **Lines 109-115** - how many open vs minimally invasive myomectomy should be mentioned in the text of the manuscript rather in just Figure 1.

Thank you, this had been added in lines 124-125: “…including 65 patients undergoing open myomectomy and 46 patients undergoing minimally invasive myomectomy.”

11. **Line 166** - how small were the number of subjects in these other studies?

This information has been added in lines 197-203: “One study assessing 7 patients undergoing abdominal myomectomy found no difference in follicle-stimulating hormone levels up to 6 months from the procedure.”
Two studies of laparoscopic myomectomy found no long-term impact on ovarian reserve as assessed by AMH, follicle-stimulating hormone levels, or antral follicle counts. One of these studies included 5 patients with follow-up data while the other included 48 patients but did not specify how many completed follow-up. These studies may have been underpowered to detect differences in markers of ovarian reserve.

12. Lines 168-171 - were there any patients that required blood transfusion? This should be mentioned.

Yes, this information has been added in lines 143-144 as well as in Table 1: “Five patients (4.5%) received a blood transfusion, of whom four underwent OM (6.2%) and one underwent MIM (2.2%, p=.32).”

13. Lines 190-195 - Does impact of blood flow affect ovarian function? What evidence is provide by this statement or is it just speculation?

The following has been added along with a discussion of AMH in lines 184-192:

“AMH is produced by the granulosa cells of growing follicles within the ovaries. While AMH is generally a stable marker of ovarian reserve, it may decline and recover in certain circumstances, such as following gonadotoxic treatments or prolonged oral contraceptive use. An insult to or suppression of growing follicles will manifest as a decrease in AMH, however as primordial follicles are recruited and grow into preantral and small antral follicles, higher serum AMH levels may be detected. Interruption in distal blood supply to the ovaries is associated with a decline in AMH levels as evidenced from studies of internal iliac artery ligation. It is possible that transient ischemia impacts growing follicles leading to a temporary decline in AMH until new follicles are recruited.”

14. Lines 200-204 - Attrition bias is understated as a limitation in the manuscript. Can the conclusion be made at 6 months post-op with just 41 patients in the analysis?

Lines 236-239 have been modified as follows: “Limitations of the study include loss of patient participation at follow-up, which may introduce bias and leads to a decreased ability to detect a significant difference in outcome at 6 months.”

15. Table 2 - should include the number of patients in each group

Number of patients in each group has been added to Table 2.

16. Figure 1 - Perhaps the discrepancy with the difference in each of the OM (40) and MIM (22) group at the 2 week postop followup accounts for the stated results?

The MIM group did have a higher percentage drop-out at 2 weeks than the OM group. As mentioned above, loss to follow-up could have introduced bias in the results.

Reviewer #3: The current manuscript presents data from a prospective cohort study to evaluate the impact of open and minimally invasive myomectomy on ovarian reserve post-operatively as measured by AMH testing. The cases were performed by a single surgeon with AMH testing pre-operatively and post-operatively at 2 weeks, 3 months and 6 months. All abdominal cases were performed with a tourniquet while laparoscopic cases utilized vasopressin.

1. Introduction, lines 40-42: Briefly state what the theorized mechanism is to decrease ovarian reserve.
Lines 46-51 have been edited as follows: “Other invasive and minimally-invasive treatments for fibroids including uterine artery embolization and hysterectomy may decrease ovarian reserve, and it is possible that myomectomy may impact ovarian reserve through similar mechanisms, such as interrupting collateral vasculature supplying the ovaries through cautery, sutures, or temporary occlusion. The use of a tourniquet to minimize blood loss during myomectomy transiently decreases blood supply to the ovaries and could potentially harm ovarian reserve.”

2. Methods, lines 75-78: Were the samples all analyzed at the same time? If not, were the study authors and surgeons aware of the pre-operative AMH?

The samples were not all analyzed at the same time, however the surgeon was not aware of the pre-operative AMH result. The following has been added to lines 77-78: “The surgeon was not aware of the pre-operative AMH level.”

3. Methods, line 82: Was vasopressin used at all during open myomectomy? Were any type of clamps or tourniquet used laparoscopically?

See lines 90-91: “Vasopressin was not routinely used during abdominal myomectomies but may have been used in rare circumstances.” And line 95: “A tourniquet was not used during MIM.”

4. Methods, line 89: Were any patients treated with oral contraceptives before or after surgery? It is known that OCPs can decrease the AMH level transiently.

Data on contraceptive use before and after surgery was not consistently available as this was generally managed by patients’ primary gynecologist. This has been added as a limitation in lines 239-240: “Data on medication such as oral contraceptives was not consistently available, as many patients returned to their primary gynecologist for long-term management.”

5. Results: It seems that when looking at the change in AMH, the mean for each group at each timeline was compared. It would be helpful to additionally evaluate the mean change in AMH per patient at each time point and also to present that with a linear graph.

The main analysis was performed based on paired comparisons. At each time point, only the patients who followed up were compared to their baseline levels. Table 2 demonstrates the results of paired analysis of mean change in AMH level.

Figure 2 demonstrates the overall mean in each group at each time point and does not represent a paired analysis. This is for visual representation but does not represent the main analysis. Patients contributed to different follow-up time points, therefore it would not be possible to plot paired means at all time points longitudinally in the same figure, and so we chose to represent the overall means here.

We did construct a spaghetti plot plotting each patient’s baseline and follow-up values, which is shown above, however due to missing values we feel this image is not as useful as figure 2.

6. Discussion: The manuscript would benefit from additional discussion of AMH itself. It is felt to be very stable over time. What pathophysiologically is thought to cause this short-term decline that is able to be recovered? What other things have been shown to cause a temporary decrease in AMH?

Thank you, the following has been added to the Discussion in lines 184-192: “AMH is produced by the granulosa cells of growing follicles within the ovaries. While AMH is generally a stable marker of ovarian reserve, it may decline and recover in certain circumstances, such as following
gonadotoxic treatments or prolonged oral contraceptive use. An insult to or suppression of growing follicles will manifest as a decrease in AMH, however as primordial follicles are recruited and grow into preantral and small antral follicles, higher serum AMH levels may be detected. Interruption in distal blood supply to the ovaries is associated with a decline in AMH levels as evidenced from studies of internal iliac artery ligation. It is possible that transient ischemia impacts growing follicles leading to a temporary decline in AMH until new follicles are recruited.”

**STATISTICAL EDITOR COMMENTS:**

*Fig 1: The loss of sample size from the initial recruitment to 2 wk, 3 mo and 6 mo follow-up makes the findings potentially biased, since < 40% of the original cohorts were included.*

This limitation of the study has been expanded on in the discussion in lines 236-239: “Limitations of the study include loss of patient participation at follow-up, which may introduce bias and leads to a decreased ability to detect a significant difference in outcome at 6 months.”

*Table 1: Gravidity and parity can only have integer values. Should format as median(range) or as categories and test with appropriate stats test. The AMH levels, EBL and fibroid wgts are formatted as mean ± SD (I assume). Need to state in Table or legend the format for the variables. Also, assuming those are means and SD, the relationship between their respective means and SDs implies skewed distributions. If the distributions were not normal, then need to either use a non-parametric test or transform the variables so that the distributions conform to normal, before applying a stats test that assumes normal distribution of the variables. Also, the SD for "All patients" fibroid wgt is too small, i.e., inconsistent with the SDs for its component subsets.*

Variables with non-parametric distributions have been changed to median (IQR) instead of mean ± SD. Variables reported as medians were compared using Wilcoxon Rank Sum, and variables reported as means were compared using Student’s t-test.

*Table 1 (continued): The groups differed at baseline in BMI etc. Why did the Authors not adjust or match to convince the reader that the groups were comparable, other than surgical approach?*

A regression analysis was performed to account for the impact of OM vs. MIM on AMH levels at each time point, adjusting for covariates including BMI, age, history of prior surgery, length of surgery, estimated blood loss, fibroid weight, and additional operative procedures.

The primary objective of the study was to assess the change in AMH level in the group overall, and was powered for paired analysis of the whole group. Differences were OM and MIM were secondary analyses. Demographics were compared between these two groups for reference in regards to subanalyses within each group. Because this was an observational prospective study, matched comparisons were not performed.

*Also, given the loss to follow-up, need to show the baseline characteristics of the cohorts at 2 weeks, 3 months and 6 months. Need to convince the reader that the pairwise analyses of those groups are representative of and generalizable to the initial group, summarized in Table 1.*

Baseline characteristics of these groups at each time point have been added in Appendix 1. See lines 145-149: “Baseline demographics among the 87 patients who presented for any follow-up, and for the 62 patients who presented at 2 weeks, the 47 patients who presented at 3 months, and the 41 patients who presented at 6 months are shown in Appendix 1. In each of these subgroups, baseline AMH level was
similar among the OM and MIM groups, and other baseline characteristics followed similar trends compared to the group as a whole.”

Table 2: Since these are paired differences, then need to cite in appropriate column how many pairs were included in each column of mean differences.

The numbers of pairs in each column have been added in Table 2.

General: While this analysis is appropriate for a descriptive series (subject to the limitation of loss of follow-up) or as comparing the two surgical approaches, it seems to lack a control group. That is, is the decrease at two weeks unique to open myomectomy, or would it also occur with other types of open abdominal surgery besides myomectomy?

It is true that the decrease in AMH level could be related to the open surgery itself. The limitation of a lack of an open control group has been added to discussion in lines 245-247: “Open surgery itself could theoretically account for the differences in post-operative AMH levels, and further study comparing different types of abdominal surgery would help to assess this factor.”

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1. If your article is accepted, the journal will publish a copy of this revision letter and your point-by-point responses as supplemental digital content to the published article online. You may opt out by writing separately to the Editorial Office, and only the revision letter will be posted.

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   * Include clinical trial registration numbers, PROSPERO registration numbers, or URLs at the end of the abstract (if applicable).
   * Name the IRB or Ethics Committee institution in the Methods section (if applicable).
   * Add any information about the specific location of the study (ie, city, state, or country), if necessary for context.

Funding information has been added to the abstract. The role of the funder has been added to the title page. The IRB institution has been named in lines 102-103.

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“Women of reproductive age” has been modified to “reproductive age people with a uterus” in line 36 and “women” has been changed to “patients” in line 42.

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7. Please add whether you received IRB or Ethics Committee approval or exemption to your Methods. Include the name of the IRB or Ethics Committee. If you received an exemption, explain why in this section.

IRB approval is included in the methods. The name of the IRB has been added in lines 102-103: “The study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai. Written informed consent was signed.”

8. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

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“Provider” has been changed to “surgeon” in line 73.

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