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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)*

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RE: Manuscript Number ONG-21-2041

Dear Dr. Edelman:

Your manuscript has been reviewed by the Editorial Board and by special expert referees. Although it is judged not acceptable for publication in Obstetrics & Gynecology in its present form, we would be willing to give further consideration to a revised version.

If you wish to consider revising your manuscript, you will first need to study carefully the enclosed reports submitted by the referees and editors. Each point raised requires a response, by either revising your manuscript or making a clear and convincing argument as to why no revision is needed. To facilitate our review, we prefer that the cover letter include the comments made by the reviewers and the editor followed by your response. The revised manuscript should indicate the position of all changes made. We suggest that you use the “track changes” feature in your word processing software to do so (rather than strikethrough or underline formatting).

Please be sure to address the Editor comments (see "EDITOR COMMENTS" below) in your point-by-point response.

Your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Nov 26, 2021, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1:

Your objective for this study is clearly stated: to determine whether doubling the dose of LNG EC improves pharmacodynamic outcomes in obese individuals, and your findings are important, adding to knowledge about EC. However, drawbacks of this study limit the strength to answer the question regarding failure of EC effectiveness in obese individuals, and whether doubling dose may impact pregnancy rates.

The major drawbacks include that your endpoint is a surrogate marker and not clinical pregnancy. There may be other variables that influence pregnancy rates. There is no control group of non-obese individuals. Without this group it is hard to draw conclusions.

Some questions to address:
Given the complex interplay between ovulation, pregnancy, the luteal phase, obesity and unknowns regarding how EC works, would there be an advantage to a RCT crossover study design in this situation? Please explain why you did not choose this methodology.

Can you provide more detail on reasons potential subjects did not meet eligibility? It would be interesting to note what the percentage of PCOS and ovulatory dysfunction was in this obese cohort because that could influence pregnancy rates in a general population of users of EC.

Clarify that confirming ovulatory status with a progesterone > 3ng/ml is an inclusion criteria and women would then be excluded if not ovulatory at baseline. That is not clear in the text or flow diagram.

It appears that there is no baseline cycle with USG and hormone monitoring. This would provide data on individual's baseline timing of follicle rupture or ovulatory dysfunction.

Half of the enrolled women have never been pregnant, and their baseline ovulatory / pregnancy success status may be relevant.

Exclusion of recent (8 week) hormonal contraception. What about DMPA which is effective for longer?

What percent of cycles had a dominant follicle?
How does a 50% delay in rupture compare to historic data on normal weight women?

Results show a difference between groups that is not statistically significant. Can you comment on whether this could be attributed to your design assumptions?

How do your results extrapolate / compare to lack of efficacy found in the clinical trials for obese women? Would you expect this level of failure or a higher level of failure? This would provide insight in the suitability of your model as a surrogate to predict clinical failure.

In line 187 you address this by saying there may be more at play such as luteal insufficiency that would impact pregnancy rates - you say prior studies show this. Please discuss this more

Line 148: "EC works by preventing LH surge, blocking follicle rupture" How robust is this theory given recent data including yours and the possibility of luteal phase insufficiency? Why did you not draw LNG levels to correlate the inhibitory threshold in this cohort?

Line 152: your prior studies show 50% decline of LNG levels in obese women. What was their concentration compared to the 0.2pg/ml threshold?

Line 157: If increasing the PK levels of LNG by doubling the dose of LNG does not work, does this put the theory of suppressing ovulation in question?

Line 169: given this alternative hypothesis of SHBG binding and obesity related differences to LNG, is it fair for you to conclude not to double the dose based on your findings? That may still achieve a clinical benefit.

Line 191: you justify not having a control group of normal weight women by saying "prior studies have shown ovulation rates similar to the rates of those in our study with standard EC dosing." Can you clarify? Are you saying standard dosing in nonobese individuals had an ovulation rate similar to those in this study meaning a delay of about 50%? Or that following this group of presumed ovulators - everyone ovulated at baseline then 50% had delayed with treatment?

Reviewer #2: Comments to the authors:

The authors present a well-designed RCT looking at different doses of levonorgestrel-containing emergency contraception (LNG EC) effect on follicular rupture > day 5 in ovulatory patients with a BMI > 30 and weight greater than 176 lbs. The objective of the study is to bridge pharmacokinetic data and clinical studies showing higher failure rates of levonorgestrel-containing emergency contraception (LNG EC) in patients with higher BMI, and current recommendations for escalating dosing for these patients.

Abstract:

Line 3 The term pharmacodynamic usually implies a dose response curve at the receptor level. The proxy for ovulation prevention including LH, progesterone, E2 levels and follicle regression are appropriate measures. The design could have benefited from a control group and a third cohort with a dose of 4.5 mg. This would, however, clearly increase the number of participants. Acknowledged in the discussion.

Line 11 I would recommend defining lack of 5 day follicle rupture as proxy for ovulation. This will make the conclusion in Line 17-18 more concise. Although most readers will understand this, as a stand-alone abstract it is not clear.

Introduction:

Line 28-30 Expand on the different mechanism of action of LNG EC vs Ulipristal acetate. The pharmacokinetics are different and blood levels for UPA may not be as impacted by BMI. Contraception 2017 May;95(5):464-469. doi: 10.1016/j.contraception.2017.01.004. Epub 2017 Jan 23.

Line 41 Clarify rapid peak level of LNG. Is it the actual peak level and or time to peak level? Cmax of Tmax.

Line 47-52 The cited pharmacokinetic studies are a good rationale for the purpose of your trial.

Methods:
Line 60 Be more specific about non hormonal forms of contraception. IUD, condoms etc. I assume progestin-based IUD would be excluded.

Line 65 How were the exclusion criteria confirmed? Was it by patient reporting only or were some base line labs done at the time of enrollment? This may be a limitation since many of the listed exclusion go under reported or diagnosed. With baseline labs many of these disorders could be identified.

Line 68-69 Define the exact times included for the luteal phase. The time would be different for someone with 21 vs 35-day cycle.

Line 74 It seems like there should be a baseline US during entry and initial progesterone testing to know what the baseline ovaries look like and is there any existing follicles or cyst that could confound subsequent cycles. What was done if the patient had more than one dominant follicle?

Line 79 Is there a reference for why >50% reduction was considered c/w ovulation?

Line 85 What hormone levels and thresholds were used to adjudicate?

Results:

Line 114-119 Having only 4 patients where definition of follicle rupture was disputed suggest good interobserver reliability. I appreciated the analysis with and without using these data points.

Table 1

Demographics would be improved adding more detailed information about baseline labs and assessment of exclusion criteria.

Figure 3

The study design does not seem to be powered for the separate per protocol analysis.

Discussion:

Line 145-146 I think the conclusion "lack of clinical efficacy" are overstated. The study would have benefited from both a placebo and larger numbers to assess the proxy more accurately being utilized for ovulation and efficacy. It would also have been helpful to include LNG levels to see if your previous data could be replicated and or adjusted for if needed.

Line 168-172 Is there an available assay that could measure SHBG?

Line 176-177 I would recommend expanding upon citation for protocols and proxy outcomes being utilized in this study. The rest of the discussion does address many of the comments mentioned in the review.

Reviewer #3:

This is a randomized trial of differing doses of oral LNG for EC in obese patients. From prior PK studies, the authors hypothesized that a double dose of LNG (3mg instead of 1.5mg) may improve EC effectiveness in obese patients. Ovulation, as detected by ultrasound and hormone levels, was used as a surrogate outcome. This research adds value to current clinical practice as we attempt to improve contraceptive options. While it is a negative study, it is important data which illustrates that PK data does not always translate to similar clinical outcomes. I selected "revise", but think only a few revisions are necessary:

-Line 55: "randomized controlled trial"-- please change to randomized trial as the study did not have a control.
-Line 76: were study staff blinded to the dose participants received? If not, did they have any contact with the participant later in the follow up process?
-Line 78: were staff performing TVUS studies blinded to the dose participants received?
-Line 78: with regards to TVUS measurements, how could you account for inter-rater reliability?
-Table 1: not knowing your individual data points for BMI, rather just a range, do you think you would have seen a different if BMI had stratified categories?
STATISTICS EDITOR COMMENTS:

Lines 10-11, 13-15: Need to explicitly state that difference in proportion of cycles with ≥ 5 day delay in follicle rupture was the primary outcome (lines 82-83).

Table 1: Should format weight as ±, not as (). All %s should be rounded to nearest integer %, not cited to 0.1% precision, since the cohorts had n = 35 each. The mean BMI was in Obese Class II, with some apparently in Obese Class III. Suggest including more information, i.e., n(%) in each Obese class in the two cohorts. Although the samples would be smaller and not the primary outcome, was there any difference in response by Obese class for the two treatment groups?

Fig 1: Were the lost to follow-up (n = 3) or other (n =2) excluded before or after randomization? That is, should were all the randomized included in analysis or were some excluded? Need to clarify ITT vs PP designation on lines 92-93 and Fig 1.

Fig 2 (K-M graph, also labelled as Figure 2): Need to include in figure legend that the p=0.2 was on the basis of log-rank test. Also, need to include along the x-axis the number in each cohort at each designated day.

Although the figures are informative, they do not directly show the comparison of the primary outcome. Need to include a short Table of relevant results, with clear separation of the primary from all secondary outcomes.

lines 197-198: That may well be true, but was not evaluated in this study.

EDITORIAL OFFICE COMMENTS:

1. The Editors of Obstetrics & Gynecology have increased transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:

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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:

   * Include your title page information in the main manuscript file. The title page should appear as the first page of the document. Add any previously omitted Acknowledgements (ie, meeting presentations, preprint DOIs, assistance from non-byline authors).
   * Funding information (ie, grant numbers or industry support statements) should be disclosed on the title page and in the body text. For industry-sponsored studies, the Role of the Funding Source section should be included in the body text of the manuscript.
   * 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.

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authorship for a submission to Obstetrics & Gynecology.” Please check with your coauthors to confirm that they received and completed this form, and that the disclosures listed in their eCTA are included on the manuscript's title page.

4. For studies that report on the topic of race or include it as a variable, authors must provide an explanation in the manuscript of who classified individuals' race, ethnicity, or both, the classifications used, and whether the options were defined by the investigator or the participant. In addition, the reasons that race/ethnicity were assessed in the study also should be described (eg, in the Methods section and/or in table footnotes). Race/ethnicity must have been collected in a formal or validated way. If it was not, it should be omitted. Authors must enumerate all missing data regarding race and ethnicity as in some cases, missing data may comprise a high enough proportion that it compromises statistical precision and bias of analyses by race.

Use "Black" and "White" (capitalized) when used to refer to racial categories. The nonspecific category of "Other" is a convenience grouping/label that should be avoided, unless it was a prespecified formal category in a database or research instrument. If you use "Other" in your study, please add detail to the manuscript to describe which patients were included in that category.

5. Clinical trials submitted to the journal as of July 1, 2018, must include a data sharing statement. The statement should indicate 1) whether individual deidentified participant data (including data dictionaries) will be shared; 2) what data in particular will be shared; 3) whether additional, related documents will be available (eg, study protocol, statistical analysis plan, etc.); 4) when the data will become available and for how long; and 5) by what access criteria data will be shared (including with whom, for what types of analyses, and by what mechanism). Responses to the five bullet points should be provided in a box at the end of the article (after the References section).

6. 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.

7. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Original Research reports should not exceed 5,500 words. Stated word limits include the title page, précis, abstract, text, tables, boxes, and figure legends, but exclude references.

8. Specific rules govern the use of acknowledgments in the journal. Please note the following guidelines:
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* 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).

* 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]."

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In addition, the abstract length should follow journal guidelines. The word limit for Original Research articles is 300 words. Please provide a word count.

11. Abstracts for all randomized, controlled trials should be structured according to the journal’s standard format. The Methods section should include the primary outcome and sample size justification. The Results section should begin with the dates of enrollment to the study, a description of demographics, and the primary outcome analysis. Please review the sample abstract that is located online here: http://edmgr.ovid.com/ong/accounts/sampleabstract_RCT.pdf. Please edit your abstract as needed.

12. 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.

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14. 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.

If appropriate, please include number needed to treat for benefits (NNTb) or harm (NNTh). When comparing two procedures, please express the outcome of the comparison in U.S. dollar amounts.

Please standardize the presentation of your data throughout the manuscript submission. For P values, do not exceed three decimal places (for example, "P = .001"). For percentages, do not exceed one decimal place (for example, 11.1\% ").

15. Please review the journal’s Table Checklist to make sure that your tables conform to journal style. The Table Checklist

16. Please review examples of our current reference style at http://ong.editorialmanager.com (click on the Home button in the Menu bar and then "Reference Formatting Instructions" document under "Files and Resources). 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 reference list.

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17. Figure 1: Please check n values for those excluded (129-60=69).

Figures 2-3: okay

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and
* A point-by-point response to each of the received comments in this letter. Do not omit your responses to the Editorial Office or Editors’ comments.

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

Again, your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Nov 26, 2021, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

John O. Schorge, MD
Associate Editor, Gynecology

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.
November 22, 2021

Dear Editor,

Please find attached our re-submission to Obstetrics & Gynecology, titled “Double dosing levonorgestrel-based emergency contraception for individuals with obesity: a randomized trial.”

We have responded to the reviewer’s comments point-by-point below in italics and have utilized track changes in the revised manuscript.

This manuscript has not been submitted to any other publication, and I do not intend to submit this manuscript to any other publication while it is under review at Obstetrics & Gynecology. A portion of this data will be presented at the EC Jamboree in October 2021 and we have submitted an abstract for consideration at the European Society of Contraception and Reproductive Health Congress in May 2022 (They do not make decisions until February 2022).

All those named in the acknowledgements have given written permission. All individuals meet criteria for authorship.

The trial was registered to clinicaltrials.gov #02859337 and received IRB approval by the Oregon Health & Science University (OHSU) IRB. The study was also conducted at EVMS and whose IRB also provided approval. Informed written consent was obtained from all participants and these are filed with other study materials.

The lead author* (below) 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.

This work was funded by the NIH. The authors designed and executed the study, analyzed the results, and prepared the manuscript.

Please contact me with any outstanding questions or concerns.

Alison Edelman, MD, MPH
Professor, OB/GYN
Director, Complex Family Planning Fellowship
Oregon Health & Science University
RESPONSE TO REVIEWER COMMENTS:

Reviewer #1:

● Your objective for this study is clearly stated: to determine whether doubling the dose of LNG EC improves pharmacodynamic outcomes in obese individuals, and your findings are important, adding to knowledge about EC. However, drawbacks of this study limit the strength to answer the question regarding failure of EC effectiveness in obese individuals, and whether doubling dose may impact pregnancy rates.

The major drawbacks include that your endpoint is a surrogate marker and not clinical pregnancy. There may be other variables that influence pregnancy rates. There is no control group of non-obese individuals. Without this group it is hard to draw conclusions.

Thank you for taking the time to critically review our manuscript. We acknowledged the limitation of using ovulation as a surrogate for pregnancy in lines 174-75 [“Our study design was based on an indirect marker of pregnancy, ovulation. However, pregnancy does not occur without ovulation.”] and listed several other limitations in our discussion (lines 182-193). We addressed other variables that could influence pregnancy rates including ovulation dysfunction. We also addressed the lack of a comparison group of non-obese women in line 192-93 (“Finally, we did not enroll a comparison group of normal BMI women. However, prior studies have shown ovulation rates similar to those seen in our study with standard EC dosing in individuals of normal BMI.[4]”) While we agree that our endpoint of ovulation based on TVUS and P4 elevation is a surrogate for pregnancy, this is a valid surrogate as ovulation must take place for pregnancy to occur.

We added the following text starting at line 182. New text in red

Our study includes the following limitations. First, we used follicle rupture as a surrogate for ovulation. Evaluation of clinical pregnancy rate, the true outcome of interest, is not feasible or ethical in a pharmacodynamic study. We confirmed ovulatory status prior to study enrollment with elevated P4 during the presumptive luteal phase, but we did not have participants undergo a baseline cycle with ultrasound and hormone monitoring. Thus, some included individuals may have unrecognized baseline ovulatory dysfunction but these individuals should have been randomly distributed between groups. We also did not evaluate the normalcy of the luteal phase during treatment cycles, as we stopped monitoring individuals as soon as rupture occurred. It is possible that abnormal ovulations and luteal insufficiency could create a difference in pregnancy rates. Prior LNG EC PD studies do combine the categories of ‘dysfunctional’ and no ovulation to demonstrate EC effectiveness but it is unclear their contribution to EC effectiveness.[4] Finally, we did not enroll a comparison group of normal
BMI women. However, prior studies have shown ovulation rates similar to those seen in our study with standard EC dosing in individuals of normal BMI. [4]

We have also added additional text regarding an NIH trial that just closed to enrollment Lines 201-203

● Some questions to address: Given the complex interplay between ovulation, pregnancy, the luteal phase, obesity and unknowns regarding how EC works, would there be an advantage to a RCT crossover study design in this situation? Please explain why you did not choose this methodology.

Thank you for these observations. We considered many study designs and settled on independent groups. Crossover studies provide each participant with two or more sequential treatments in a random order usually separated by a washout period, and allow each participant to act as her own control. A disadvantage is that the crossover design is challenging when the condition of interest is unstable. Cycle length and ovulation may vary over the course of several cycles of observation. It is also challenging to retain participants willing to have repeated intensive interventions over multiple cycles (baseline, treatment, washout, treatment).

We added the following text in red to the discussion (Line 174) “Strengths of our study include the randomized design with independent groups and rigorous assessment of outcomes. While a crossover design allows each participant to serve as her own control, we have found it difficult to retain participants willing to undergo repeated intensive intervention over multiple cycles (baseline, treatment, washout, treatment).

● Can you provide more detail on reasons potential subjects did not meet eligibility? It would be interesting to note what the percentage of PCOS and ovulatory dysfunction was in this obese cohort because that could influence pregnancy rates in a general population of users of EC.

Thank you for this comment. We have added the major categories for exclusion to CONSORT Figure 1.

Individuals could not hold a prior diagnosis of PCOS (either self or clinically diagnosed from record review), we perform a clinical exam at the time of screening and individuals could not have any evidence of androgen excess nor would they be eligible if they did not have regular cycles. We did not formally screen subjects for PCOS with specific laboratory tests as PCOS is a diagnosis of exclusion. We have added the following to Line 60-63 (red text) “Subjects were required not to be at risk for pregnancy (abstinent or using a non-hormonal method of contraception, e.g. pills/patches/rings, injection, hormonal IUD). Major exclusion criteria obtained via patient report, review of medical records,
and clinical exam included: metabolic disorders including uncontrolled thyroid dysfunction and polycystic ovarian syndrome or clinical evidence of androgen excess; impaired liver or renal function; actively seeking or involved in a weight loss program (must be weight stable); pregnancy, breastfeeding, or seeking pregnancy; recent (8 week) use of hormonal contraception; current use of drugs that interfere with metabolism of sex steroids; smoking or vaping; chronic marijuana use.

- Clarify that confirming ovulatory status with a progesterone > 3ng/ml is an inclusion criteria and women would then be excluded if not ovulatory at baseline. That is not clear in the text or flow diagram.

**New Text (Lines 67-69) has been added in order to clarify that P >3ng/mL is an inclusion criteria.**

“Subjects underwent an initial phone screen and then if eligible, completed an in-person screening visit to collect baseline demographic, health information, and a serum progesterone level during luteal phase to confirm ovulatory status (progesterone level ≥3 ng/mL), an inclusion criterion for participation.”

- It appears that there is no baseline cycle with USG and hormone monitoring. This would provide data on individual’s baseline timing of follicle rupture or ovulatory dysfunction.

**We started monitoring individuals early enough in the cycle in order to capture follicle development and hormone data but we agree that this is a weakness of the study, and have already acknowledged this in the discussion (lines 187-189).**

“We confirmed ovulatory status prior to study enrollment with elevated P4 during the presumptive luteal phase, but we did not have participants undergo a baseline cycle with ultrasound and hormone monitoring”. No changes made.

- Half of the enrolled women have never been pregnant, and their baseline ovulatory / pregnancy success status may be relevant.

**Although we appreciate the comment, we believe that pregnancy success is beyond the scope of this pharmacodynamic study. No changes made.**

- Exclusion of recent (8 week) hormonal contraception. What about DMPA which is effective for longer?

**Thank you for the comment. We excluded all hormonal contraception. Prior users of DMPA needed to have returned to regular cycles and have demonstrated return to ovulation (P>3 ng/mL). No changes made.**

- What percent of cycles had a dominant follicle?

**Participants were not randomized to receive study drug if they did not develop a qualifying dominant follicle. Line 74-77. No changes made. We have included**
some additional information in the CONSORT figure that does address this issue. Two individuals did not develop a dominant follicle with monitoring.

● How does a 50% delay in rupture compare to historic data on normal weight women? Line 201 We have noted that prior ovulation rates are similar in PD studies of normal BMI individuals.

● Results show a difference between groups that is not statistically significant. Can you comment on whether this could be attributed to your design assumptions?

The methods provides or power analysis (Lines 90-93). Our sample size had 80% power to determine a 30% difference if follicle rupture between groups at a 5% significance level. We considered this effect size clinically-important, and cannot comment on smaller effect sizes. No changes made.

● How do your results extrapolate / compare to lack of efficacy found in the clinical trials for obese women? Would you expect this level of failure or a higher level of failure? This would provide insight in the suitability of your model as a surrogate to predict clinical failure.

Thank you for these comments. As pointed out in the introduction, we undertook this study to determine whether double doses could improve effectiveness of LNG EC based on the observed failures seen in clinical trials. As pointed out in the discussion, we did not see a PD difference. Further evaluation of clinical pregnancy will require a clinical trial. No changes made.

● In line 187 you address this by saying there may be more at play such as luteal insufficiency that would impact pregnancy rates - you say prior studies show this. Please discuss this more

Thank you for the comment. We believe that further discussion of infertility mechanisms in obese women are outside the scope of the paper. Ref 4 is an example of a prior EC study which combines both ovulation and luteal dysfunction rates to calculate the potential impact on pregnancy rates. No changes made.

● Line 148: "EC works by preventing LH surge, blocking follicle rupture" How robust is this theory given recent data including yours and the possibility of luteal phase insufficiency? Why did you not draw LNG levels to correlate the inhibitory threshold in this cohort?

Prior Data strongly support ovulation inhibition as the mechanism of progestins and these PD studies are supported by pregnancy trials. We looked at LNG levels in a prior study. We agree that it would have been helpful to recheck PK parameters but we could not justify the invasiveness or cost of these given the
clear results of our prior PK study. Single LNG levels while useful for compliance if not performing direct observation of intake, which we did, are not useful for determining or confirming hypotheses.

● Line 152: your prior studies show 50% decline of LNG levels in obese women. What was their concentration compared to the 0.2pg/ml threshold?
We don’t know the level for EC, as it relates to time to achieve the level

Line 157: If increasing the PK levels of LNG by doubling the dose of LNG does not work, does this put the theory of suppressing ovulation in question?

● Line 169: given this alternative hypothesis of SHBG binding and obesity related differences to LNG, is it fair for you to conclude not to double the dose based on your findings? That may still achieve a clinical benefit.

We are not aware of an alternative hypothesis of SHBG binding affecting LNG PK/PD as presented by your comment. Given that obese women typically have lower SHBG – their active drug levels should be higher or unbound but that is not resulting in the expected PD outcomes. No changes made.

● Line 191: you justify not having a control group of normal weight women by saying "prior studies have shown ovulation rates similar to the rates of those in our study with standard EC dosing." Can you clarify? Are you saying standard dosing in nonobese individuals had an ovulation rate similar to those in this study meaning a delay of about 50%? Or that following this group of presumed ovulators - everyone ovulated at baseline then 50% had delayed with treatment?

We designed our study to compare PD in an obese population. Historical non-obese data is available for the majority of the prior EC studies that brought LNG EC to market. However, it is always nice to have a control and we acknowledge that a non-obese control would have added additional data, but funding is not limitless and we did not have funding for all experiments that would have been desirable.

Reviewer #2:

● The authors present a well-designed RCT looking at different doses of levonorgestrel-containing emergency contraception (LNG EC) effect on follicular rupture > day 5 in ovulatory patients with a BMI > 30 and weight greater than 176 lbs. The objective of the study is to bridge pharmacokinetic data and clinical studies showing higher failure rates of levonorgestrel-containing emergency contraception (LNG EC) in patients with higher BMI, and current recommendations for escalating dosing for these patients.

Thank you.
Abstract:
●Line 3  The term pharmacodynamic usually implies a dose response curve at
the receptor level. The proxy for ovulation prevention including LH,
progesterone, E2 levels and follicle regression are appropriate measures. The
design could have benefited from a control group and a third cohort with a dose
of 4.5 mg. This would, however, clearly increase the number of participants.
Acknowledged in the discussion.

See response for reviewer 1 regarding control groups and additional studies.

●Line 11  I would recommend defining lack of 5 day follicle rupture as proxy for
ovulation. This will make the conclusion in Line 17-18 more concise. Although
most readers will understand this, as a stand-alone abstract it is not clear.

The lack of ovulation for 5 days represents two different important concepts –
sperm remain viable for 5 days in the reproductive tract and then also LNG
inhibits ovulation. We agree that 'delay' may be confusing as this relates to
more of a UPA EC mechanism that delays follicle rupture rather than inhibits so
we have tried to clarify this throughout the manuscript (including methods and
discussion). We have also added clarification from the Journal’s Stats Editor in
the abstract for Line 11 regarding the primary outcome. See track changes.

Introduction:
●Line 28-30 Expand on the different mechanism of action of LNG EC vs Ulipristal
acetate. The pharmacokinetics are different and blood levels for UPA may not
be as impacted by BMI. Contraception 2017 May;95(5):464-469. doi:

We do feel like getting into the differences between LNG and UPA would be out
of scope for this manuscript. We mention UPA purely to be comprehensive
regarding identifying the oral agents but otherwise the differences in
pharmacokinetics is beyond the scope of this research. No changes made to
manuscript. We utilize this reference later in the introduction as it also looked at
LNG.

●Line 41 Clarify rapid peak level of LNG. Is it the actual peak level and or time to
peak level? Cmax of Tmax.

Great point, no one actually knows if it is time to peak or the actual peak or both
which is why we kept our statement general. No changes made.

●Line 47-52  The cited pharmacokinetic studies are a good rationale for the
purpose of your trial.

Thank you.
Methods:

● Line 60  Be more specific about non hormonal forms of contraception. IUD, condoms etc. I assume progestin-based IUD would be excluded.

Correct anything hormonal including a hormonal IUD. Change made to text Line 60. Examples included.

● Line 65  How were the exclusion criteria confirmed? Was it by patient reporting only or were some base line labs done at the time of enrollment? This may be a limitation since many of the listed exclusion go under reported or diagnosed. With baseline labs many of these disorders could be identified.

See response for reviewer 1.

● Line 68-69  Define the exact times included for the luteal phase. The time would be different for someone with 21 vs 35-day cycle.

We added the additional text line 69 “based on an individual’s cycle length”.

● Line 74  It seems like there should be a baseline US during entry and initial progesterone testing to know what the baseline ovaries look like and is there any existing follicles or cyst that could confound subsequent cycles. What was done if the patient had more than one dominant follicle?

Initial progesterone testing was performed during screening. In order to limit invasive procedures to study participants, If at the initiation of TVUS monitoring they had ovarian abnormalities or ovaries could not be easily visualized, then early withdrawal/discontinuation would occur. All follicles were followed and noted. No changes made.

● Line 79  Is there a reference for why >50% reduction was considered c/w ovulation?

We utilized the standard definition utilized in EC research which is >50% collapse or disappearance. We have added references to the statement.

● Line 85  What hormone levels and thresholds were used to adjudicate?

Adjudication is determined based on pattern of hormones before evidence of collapse (rise from baseline) and then also levels. Rupture had to be preceded by a rise in estradiol and LH. Progesterone elevation above 3 is not always seen because visits might stop before luteal phase starts.

Results:
• Line 114-119 Having only 4 patients where definition of follicle rupture was disputed suggest good interobserver reliability. I appreciated the analysis with and without using these data points.

Thank you. We think its important to be transparent about how this variability might impact the results. It did not but there is always the chance that it could. No changes made.

Table 1
• Demographics would be improved adding more detailed information about baseline labs and assessment of exclusion criteria.
See reviewer 1 response regarding exclusion criteria, baseline labs and CONSORT FLOW Fig 1.

Figure 3
• The study design does not seem to be powered for the separate per protocol analysis.

This figure represents the ITT analysis. No changes made except to correctly label this figure as Figure 3 (instead of Figure 2).

Discussion:
• Line 145-146 I think the conclusion “lack of clinical efficacy” are overstated. The study would have benefited from both a placebo and larger numbers to assess the proxy more accurately being utilized for ovulation and efficacy. It would also have been helpful to include LNG levels to see if your previous data could be replicated and or adjusted for if needed.

We agree that our study cannot state ‘lack of clinical efficacy’. We have tried not to be explicit regarding this ‘jump’ but at the same time we do feel some obligation to address the clinical implications. Unfortunately, our earlier PK studies of only TEN individuals (5 normal BMI and 5 obese BMI) has been used by both clinical guidance agencies and clinicians as evidence to clinically recommend doubling the dose. We would like to temper that enthusiasm based on our findings and thus have tried to fine the right balance in which to do so. While our findings cannot definitively close the door on the double dosing issue, they do provide information that maybe more than just a higher dose is needed.

We agree that it would have been helpful to recheck PK studies but we could not justify the invasiveness or cost of these given the clear results of our prior PK study.

• Line 168-172 Is there an available assay that could measure SHBG?

Yes, SHBG assays are available and this is something we studied in our prior PK publication. No changes made.
I would recommend expanding upon citation for protocols and proxy outcomes being utilized in this study. *We have clarified this issue also raised by reviewer 1’s.*

The rest of the discussion does address many of the comments mentioned in the review.

*Thank you. No changes made.*

**Reviewer #3:**

This is a randomized trial of differing doses of oral LNG for EC in obese patients. From prior PK studies, the authors hypothesized that a double dose of LNG (3mg instead of 1.5mg) may improve EC effectiveness in obese patients. Ovulation, as detected by ultrasound and hormone levels, was used as a surrogate outcome. This research adds value to current clinical practice as we attempt to improve contraceptive options. While it is a negative study, it is important data which illustrates that PK data does not always translate to similar clinical outcomes. I selected "revise", but think only a few revisions are necessary:

- Line 55: "randomized controlled trial"— please change to randomized trial as the study did not have a control.
  *We appreciate that the study would have been enhanced by a second control group (a normal BMI control group). However, this study did have an obese control group – one that received the current standard treatment of LNG 1.5mg. No changes made.*

- Line 76: were study staff blinded to the dose participants received? If not, did they have any contact with the participant later in the follow up process?
  *Neither participant nor study staff were blinded to dose. A small group of trained study staff were involved with study procedures. The daily CRFs did not denote treatment allocation. Objective, not subjective, outcomes were obtained for the primary endpoint. An investigator masked to treatment allocation reviewed the data. No changes made.*

- Line 78: were staff performing TVUS studies blinded to the dose participants received?
  *See above*

- Line 78: with regards to TVUS measurements, how could you account for inter-rater reliability?
All investigators performing TVUS measurements were trained and only a small number of investigators participated in the study. An investigator masked to treatment allocation reviewed the data Line 89-90. No changes made.

- Table 1: not knowing your individual data points for BMI, rather just a range, do you think you would have seen a different if BMI had stratified categories?

We were not powered to stratify BMI categories beyond the inclusion criteria of a BMI 30kg/m2.

**STATISTICS EDITOR COMMENTS:**

Lines 10-11, 13-15: Need to explicitly state that difference in proportion of cycles with ≥ 5 day delay in follicle rupture was the primary outcome (lines 82-83).

We assume it is ok to go beyond the standard abstract word count. We have added “Our primary outcome was the difference in the proportion of subjects with a delay in follicle rupture of 5 days post-dosing (yes/no) between groups” to the text line 10.

Table 1: Should format weight as ±, not as (). All %s should be rounded to nearest integer %, not cited to 0.1% precision, since the cohorts had n = 35 each. The mean BMI was in Obese Class II, with some apparently in Obese Class III. Suggest including more information, i.e., n(%) in each Obese class in the two cohorts. Although the samples would be smaller and not the primary outcome, was there any difference in response by Obese class for the two treatment groups?

We have corrected Table 1 as requested. We have utilized track changes in the revised version. We were not powered to stratify BMI categories beyond the inclusion criteria of a BMI 30kg/m2 but we have added this additional demographic information into Table 1.

Fig 1: Were the lost to follow-up (n = 3) or other (n =2) excluded before or after randomization? That is, should were all the randomized included in analysis or were some excluded? Need to clarify ITT vs PP designation on lines 92-93 and Fig 1.

Participants were not randomized to receive study drug if they did not develop a qualifying dominant follicle. Line 74-77. No changes made. We have included some additional information in the CONSORT figure that does address this issue. The CONSORT notes the ITT. Should we specify that in the Figure title or text or both?
Fig 2 (K-M graph, since there is a histogram, also labelled as Figure 2): Need to include in figure legend that the p=0.2 was on the basis of log-rank test. Also, need to include along the x-axis the number in each cohort at each designated day.

We have corrected the figure legend with the recommended text. We have also removed Figure 2 (bar graph) as it seems to highlight the secondary outcome too much; thus we changed the K-M figure to Figure 2. We have also designated the number in each cohort for each day on the x-axis (not in track changes).

Although the figures are informative, they do not directly show the comparison of the primary outcome. Need to include a short Table of relevant results, with clear separation of the primary from all secondary outcomes.

We actually deliberated this specific issue with the authorship team prior to the original submission but the small table of the primary results seemed extremely duplicative with the text as it would look like this:

<table>
<thead>
<tr>
<th></th>
<th>LNG 1.5 mg</th>
<th>LNG 3.0 mg</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 day or greater delay in rupture</td>
<td>18/35 (51%)</td>
<td>24/35 (69%)</td>
<td>0.14*</td>
</tr>
</tbody>
</table>

* Chi-square test

We have not yet added this table to the revision but instead edited the text in the results section and removed the Figure 2 Bar Graph to aid in the delineation of the primary and secondary outcomes.

● lines 197-198: That may well be true, but was not evaluated in this study.

We agree which is why we clearly identified that we did not evaluate it in this study but felt like it was important to note it. We are unsure if the editor would like us to change this sentence based on their comment? No changes made.