OBSTETRICS & GYNECOLOGY

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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.*

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Questions about these materials may be directed to the *Obstetrics & Gynecology* editorial office: obgyn@greenjournal.org.

Date: Mar 05, 2020
To: "Intira Sriprasert" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-20-262

RE: Manuscript Number ONG-20-262

Determinants of serum estradiol levels among postmenopausal women using hormone therapy

Dear Dr. Sriprasert:

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).

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 Mar 26, 2020, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1: The authors of this manuscript present a post hoc analysis of the ELITE trial, a single-center, randomized, double blinded, placebo controlled trial of HT in healthy postmenopausal women stratified to early or late menopause. The stated objective of the study was to explore determinants of estradiol (E2) levels among study participants.

The citation describing the utility of measuring serum estradiol levels (Yasui, et al, 2001) was a small study evaluating different estradiol levels for different clinical endpoints. This is a small study with 34 patients in each of two arms receiving different doses of HT than used in this study. Measuring serum E2 levels is unfamiliar to most obstetrician gynecologists prescribing hormone therapy in the postmenopause as they do so for menopausal symptoms and titrate based upon symptoms. A more robust description of the findings of the authors previous work associating E2 levels and atherosclerosis would be more appropriate for the readership of this journal. Specific E2 levels are not currently guideline recommended within clinical care. When measured serum E2 levels 40-100 pg/mL are considered a reasonable target - is this consistent with the authors recommendations?

Are there harms associated with higher serum E2 levels in either the later or early menopausal patient population. Since E2 levels are infrequently drawn in clinical practice these are not well described in the literature but are relevant to the topic of this manuscript.

Another issue with serum estradiol levels is levels peak and trough during the day. Were estradiol levels checked at the same time of day? Was this variability accounted for in any of the results? Across trial variability was not described. Did patients E2 levels change during the time they were followed on study?

The discussion nicely summarizes the factors which influence E2 levels - BMI, Alcohol, Smoking, Antifungal medicine, but does not summarize how these findings are important to a prescriber. Should doses be modified based upon these factor? 1mg daily of estradiol is a dose higher than is typically used in postmenopausal HT - do these factors warrant dose modifications for women using MHT?

In Tables 1 and 2, Hispanic is listed as a category for race. Recommend using census terms and ethnicity. https://www.census.gov/mso/www/training/pdf/race-ethnicity-onepager.pdf

In Table 2, would recommend including the E2 values for each group.

Reviewer #2:

Precis - BMI, surgical menopause, alcohol use, smoking, and antifungal medications are determinants of serum estradiol levels among postmenopausal women on HRT

Abstract - Objective: benefit of HT - atherosclerosis related to E2 levels - identify determinants of E2 on healthy women on HT
Methods - women from ELITE trial RCT - 1 mg E2 +/- vaginal progesterone with 80% compliance; E2 measured q 6 mo over median 4.8 yrs; demographics, clinical characteristics, medication use, biomarkers of liver/kidney function all collected
Results - 275 women - avg age / time to menopause in early vs late menopause - 55.4 vs 64.4 yr and 3.6 versus 16 years adjusted for pretreatment E2 - increased E2 if higher BMI, higher creatinine, surgical menopause, etoh use and antihypertensive medications; smoking and antifungal use was associated with lower E2
multivariable model - increased BMI, surgical menopause, and alcohol associated with higher E2 and smoking and antifungals were associated with lower E2; the determinants were the same for early versus late menopause
Conclusions - determinants for E2 - BMI, surgical menopause, etoh, smoking, antifungal medications; E2 relates to treatment efficacy and most HT determinants are modifiable; the desirable E2 levels can be obtained through personal intervention

Introduction - E2 levels are different for different treatment endpoints - reviewed; HT has decreased risk coronary heart disease/atherosclerosis in early menopause but no affect in late menopause; ELITE trial findings - decrease atherosclerosis with E2 in early menopause but increased in late; goal is to explore determinants of E2 levels

Methods - study design - post-hoc analysis of ELITE RCT - < 6 yr after menopause vs > 10 yr; 1 mg E2 +/- vaginal progesterone - followed between 1/05-2/13 for avg 4.8 yr
primary results - HT - decreases progression of atherosclerosis when initiated early but not late
80% compliance by tablet count to be evaluated; serum E2 measurements at baseline and q 6 months
Measurement E2 correlates - race, type of menopause, vitamins/ fish oil, weight, BMI, smoking, Etoh, exercise, medications, kidney and liver function - assessed q 6-12 months
Statistical analysis - described

Results - 275 women with adequate compliance - 123 with early menopause and 152 with late
baseline characteristics - age 55 vs 64 time since menopause 3.6 yr vs 16 yr
Serum E2 - baseline - early and late was the same - approx. 10; on trial E2 mean and median was the same between early and late menopause - approx 52-56
association between log transformed E2 and potential correlates - increased E2 associated with BMI, surgical menopause, Etho and antihypertensive medications; decreased E2 associated with smoking and antifungal meds
past smokers had lower E2 and current smokers had the lowest E2; Etoh had increased E2 - other factors were not associated
Multivariable association log transformed E2 - elevated E2 associated with BMI and etoh use
surgical menopause - increased E2 borderline statistically significant
lower E2 with smoking and antifungals
determinants of E2 similar between early and late menopause

Discussion - BMI, surgical menopause, ETOH use, smoking, and antifungal medications had statistically significant correlation with E2 levels - same between early and late menopause
the association with alcohol is stronger with early menopause and biological explanation involves E2 metabolism
BMI - increased BMI leads to increased E2 through peripheral aromatization
ETOH - biological rationale reviewed
smoking - increased hepatic metabolism with oral E2 but not transdermal and decreased benefit of symptoms with smokers
antifungal meds -impact on metabolism
nonsignificant variables - other medications and exercise
Strengths/ limitations - RCT data, determinants of E2 level in women with good compliance but measures total E2 and not other metabolites
surgical menopause - no explanation and small group - 11%

Public health/ conclusions - benefits of HT on atherosclerosis related to E2 levels and significant determinants of E2 - weight, etoh, smoking - are modifiable
consider control of weight, smoking, etoh in achieving lowest effective dose with minimum side effects for each patient

Comments -

This is overall a well-written good analysis of statistics and important to see the effect of various factors on estradiol levels with HT.

1) Statistics should be reviewed by a statistician

2) Not sure there are enough patients with surgical menopause to list this as a criteria given that there are few patients and data is only borderline, and there is no real biological plausibility

3) Perhaps mention of threshold of alcohol use should be mentioned as it appears that it is only relevant in women with use > 2 drinks per day so mild alcohol use was not a determinant

Reviewer #3: The statement "E2 levels are associated with HT treatment efficacy" (line 79 and again line 318) is vague and not supported with a reference. This statement, which is the crux of the author's conclusion, is heavily dependent on how the author defines "efficacy." Is efficacy control of vasomotor symptoms? Reducing risk of osteoporosis-related fractures? Alteration of lipid profile?

Trying to figure out what "efficacy" means in the context of this research, I was unable to find evidence that ties E2 level to patient perception of vasomotor symptom relief. Yasui (2001), which you cited, actually states that the Kupperman index (a questionnaire of vasomotor symptoms) is not related to serum estradiol level. Bone loss does appear to be reduced in HT users and there appears to be conflicting information about carotid intima-media thickening in young HT users. And while some markers of lipid profile like HDL and LDL do improve on oral estrogen, others like triglycerides do not, so I don't think this is what is meant by "efficacy" in the context of this research.

Since your conclusion states that important public health implications stem from your statement that "achieved E2 levels relate to the HT treatment efficacy," I think a more specific description of what you mean by "efficacy" is vital. Otherwise, the concept of efficacy is left up to the reader and they may draw a conclusion (related to vasomotor symptom relief, for example) that is not actually supported by your research. Something along the lines of "achieved E2 levels relate to the HT treatment biological effect" rather than efficacy seems more accurate and less confusing.

You referenced Ginsburg JAMA 1996 which described the relationship between alcohol consumption and serum estradiol level. Your research provides a much larger sample size which supports that earlier finding. So while this finding is not new, it is potentially helpful to see this finding supported by a larger sample size.

In the paragraph "Associations between log-transformed on-trial E2 levels and potential correlates" (line 196) that follows with the statement "Adjusted for baseline serum E2 level..." you mention that creatinine is "not significantly associated with serum E2 levels." However, in the results section of your abstract, you list higher creatinine being associated with higher serum E2 levels (line 54). This appears to be conflicting information. I don't see anywhere in the rest of the article documenting any association with E2 level and creatinine. If it is a discrepancy, it should be corrected. If it is not a discrepancy, it might be worthwhile clarifying in the body of the article exactly how a positive relationship between E2 and creatinine WAS found. It seems confusing to me and clarifying it might help me and other readers.

Overall, the article further supports the idea that improving modifiable lifestyle factors such as weight, smoking and alcohol use are important (which is already supported by ample other evidence in the literature). While these recommendations are not novel, being able to better educate patients that are taking or considering HT is likely helpful.

Reviewer #4: This is a well-written manuscript with succinct text and excellent display of the data. The topic is important and has implications for clinical practice. I have a few comments.

1) The authors confidently state that serum E2 levels < 15, >=15 and >= 25 pg/ml are associated with different tissue effects, but this is based upon a single 2001 reference (Reference 3) with only 25 women in the < 15 group, 27 women in the >=15 to < 25 group and 16 women in the >=25 group. That article suggests in the discussion that low concentrations of estradiol (< 15 pg/ml) was sufficient to suppress LH and FSH "and to relieve VMS", but the article shows no results for VMS. Please provide reference for estradiol and decrease in VMS and additional references to support thresholds for symptom and tissue effects of serum estradiol.

2) The authors state mean serum E2 level achieved was 46.5 (30.6) pg/ml, range 5 to 330 pg/ml in their trial population(5). Please comment on this wide range in this select population of adherent women taking E2. Or is this among adherent and nonadherent women and if so, only provide range for adherent women. If this is for adherent women, why is the range so wide?

3) Please comment on when E2 serum samples were obtained in relation to the 4% vaginal micronized progesterone administration and why or why not this might matter.

4) Methods, In your tables and text, did I miss the list the covariates in the models that were included?

5) How did you account for repeated measures, i.e. you had 2160 E2 measures in 275 women.

6) I was surprised by similar baseline E2 levels between early and late menopause participants. How do you explain this?

7) Your explanation for higher E2 levels among surgically menopause women, "a unique population sampling" is a single explanation of your findings, but please remind the reader only 30 women had surgical menopause with ? 15 women each in the early and late postmenopausal groups? More likely explanation of these findings is existing confounding that is not accounted for, like obesity or alcoholism or other. Please comment.

8) Why would past smoking affect serum E2 levels, and in addition, why was this observed only in your younger women (early postmenopausal)?

9) Only 2 women used antifungals at baseline, yet there are 45 samples in which women were using antifungals, is this correct? Please discuss as limitation.

10) Line 312, there is an extra "the".

11) Can you comment in discussion about serum E2 and race (SWAN references)?

12) Only 9 women had > 2 drinks per day, what was the breakdown by early and late menopause? Please discuss as limitation.

13) Discussion on ETOH - you show differences in mean E2 levels between individuals with no ETOH, < 1 ETOH and 1-2 drinks. Would it be fair to more explicitly recommended less than <1 drink per day in your conclusions until a study can be done with more numbers? Please comment.

14) Your conclusion, "Postmenopausal women should control their weight and refrain from smoking and alcohol use with the goal of obtaining the lowest effective dose of HT," is fair but rather vague. More explicitly, should you suggest, women who are obese and cannot lose weight and who drink more than 1 drink per day who are 10 years from menopause may be at higher risk for atherosclerosis due to higher estrogen levels or if taking HT, lower doses may be sufficient for optimal tissue effects. And, women who cannot stop smoking may need more estrogen to attain adequate serum estrogen levels and to decrease fracture risk.

15) Not all would agree with your statement, "In particular, we have previously reported that the beneficial effect of HT on atherosclerosis progression among postmenopausal women is related to achieved serum E2 levels.(6)" Please temper this statement by referencing studies that do not agree with your prior findings, or qualify.

STATISTICAL EDITOR COMMENTS:

The Statistical Editor makes the following points that need to be addressed:

Table 2: The "n" for subsets (eg, smoking status: never, past or current, alcohol use, etc) should include the number of women in those categories, in addition to the number of measurements. There are multiple comparisons in this Table, with no adjustment for multiple comparisons. Some of the comparisons are likely spurious (eg, surgical vs natural menopause, highest tertile of weekly hrs of physical activity, use of antihypertensives or antifungals (additional issue is the sample of only 2 women for antifungal use, insufficient to infer general conclusions re: E2 levels from these data) and smoking status or alcohol use, which only achieve statistical significance by p-trend, by individual categories, were either NS or marginally so. Also, the formatting of ß and their SE by log, rather than by transformed values, is not very useful for clinicians. Should include the log values as on-line supplemental, and use transformed values in main text.

Table 3: Need to include the "n" for each subset (number of women and number of measurements) when stratified by early vs late menopause. Since the antifungal use had N = 2 women, either there was one woman in each category of early vs late or the values were derived by the model, inferring the effect of antifungal use in each stratum. In any case, the number of women using antifungals is insufficient to include in a general model of E2 levels. Should omit from the model. Also, although the overall p-value trends for E2 level vs alcohol use were significant for the total and for the early menopause groups, when compared categorically, only the highest intake level was actually significantly associated with higher E2 levels. Therefore, the general conclusion that E2 is associated with alcohol intake should be clarified, since it only applied to analysis of the highest intake group. That group had n = 9 women with only 45 measurements and the reader cannot tell how many of those were in the early vs late stratum. Extrapolating from those relatively sparse data to a general conclusion about alcohol intake vs E2 seems imprecise. In summary, the conclusion from Table 3 is an

association of E2 with BMI, but not surgical vs natural menopause, a negative association between E2 and smoking status, but only for the early menopause stratum and associations of E2 vs the highest level of alcohol use or the use of antifungals, which were based on small samples and may not be reproducible.

lines 63, 164-170, 214-215: Borderline is actually NS by the threshold cited.

lines 262-278 and Tables 2, 3: How do these mechanisms account for the association of E2 levels vs past smokers?

EDITOR'S COMMENTS

We no longer require that authors adhere to the Green Journal format with the first submission of their papers. However, any revisions must do so. I strongly encourage you to read the instructions for authors (the general bits as well as those specific to the feature-type you are submitting). The instructions provide guidance regarding formatting, word and reference limits, authorship issues, and other things. Adherence to these requirements with your revision will avoid delays during the revision process, as well as avoid re-revisions on your part in order to comply with the formatting. For instance, we don't use headings such as "Study design" in the different paper sections.

Line 33: "Determine" seems like too strong a causal word here. Based on your data, it seems that "influence" may be better. None of these are deterministic. Similar wording, used elsewhere such as line 62 and line 64, should be edited. Rather than Determinants, perhaps "factors" or something similar.

In the abstract, please provide the standard deviations.

PRESENTATION OF STATS INFORMATION
P Values vs Effect Size and Confidence Intervals: While P values are a central part of inference testing in statistics, when cited alone, often the strength of the conclusion can be misunderstood. Whenever possible, 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.

This is true for the abstract as well as the manuscript, tables and figures.

Please provide absolute values for variables, in addition to assessment of statistical significance.

We ask that you provide crude OR's followed by adjusted OR's for all relevant variables.

The objective of the abstract should be a simple "To" statement without background information.

Line 71: Maybe used "by" postmenopausal women?

Line 92: in the 2018 NASM statement about hormone therapy, the recommendation is to individual treatment formulation, dose and route to achieve desired end but from my review of this document, there is no recommendation to follow serum levels in this management recommendation. It seems appropriate for you to at least acknowledge this either here or in the discussion section, preferably in the introduction in order to put this into context for the reader. It seems prudent to make it clear that you are not recommending following serum E2 levels.

Line 96: again, determinants.

Line 102: was this a planned post hoc analysis? I really appreciate your clear description of the original trial and the outcomes.

Line 199 Please limit p values to 3 decimal places.

Line 215: We do not allow authors to describe variables or outcomes in terms that imply a difference (such us of the terms "trend" or "tendency" or "marginally different") unless there is a statistical difference. Please edit here and throughout to indicate that there is no difference.

Line 245: please avoid causal language here. This is an association. Please edit this language here and where ever it occurs in the report.

Line 279 Please note statistical reviewer comments regarding caution with respect to anti fungal data interpretation, given the very small number of exposed patients.

Line 322: This is a rather narrow goal for recommending women control their weight, refrain from smoking and limit

alcohol use. Perhaps achieving the lowest dose of HT could be one goal for these overall health goals but not THE goal?

EDITORIAL OFFICE COMMENTS:

1. The Editors of Obstetrics & Gynecology are seeking to increase 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:
A. OPT-IN: Yes, please publish my point-by-point response letter.
B. OPT-OUT: No, please do not publish my point-by-point response letter.

2. As of December 17, 2018, Obstetrics & Gynecology has implemented an "electronic Copyright Transfer Agreement" (eCTA) and will no longer be collecting author agreement forms. When you are ready to revise your manuscript, you will be prompted in Editorial Manager (EM) to click on "Revise Submission." Doing so will launch the resubmission process, and you will be walked through the various questions that comprise the eCTA. Each of your coauthors will receive an email from the system requesting that they review and electronically sign the eCTA.

Please check with your coauthors to confirm that the disclosures listed in their eCTA forms are correctly disclosed on the manuscript's title page.

3. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript's lead author. The statement is as follows: "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." *The manuscript's guarantor.

If you are the lead author, please include this statement in your cover letter. If the lead author is a different person, please ask him/her to submit the signed transparency declaration to you. This document may be uploaded with your submission in Editorial Manager.

4. The Editors believe this secondary analysis should follow STROBE guidelines. If you believe this is an incorrect assessment, please let us know which guideline you intend to follow in your revision's cover letter.

Responsible reporting of research studies, which includes a complete, transparent, accurate and timely account of what was done and what was found during a research study, is an integral part of good research and publication practice and not an optional extra. Obstetrics & Gynecology supports initiatives aimed at improving the reporting of health research, and we ask authors to follow specific guidelines for reporting randomized controlled trials (ie, CONSORT), observational studies (ie, STROBE), meta-analyses and systematic reviews of randomized controlled trials (ie, PRISMA), harms in systematic reviews (ie, PRISMA for harms), studies of diagnostic accuracy (ie, STARD), meta-analyses and systematic reviews of observational studies (ie, MOOSE), economic evaluations of health interventions (ie, CHEERS), quality improvement in health care studies (ie, SQUIRE 2.0), and studies reporting results of Internet e-surveys (CHERRIES). Include the appropriate checklist for your manuscript type upon submission. Please write or insert the page numbers where each item appears in the margin of the checklist. Further information and links to the checklists are available at http://ong.editorialmanager.com. In your cover letter, be sure to indicate that you have followed the CONSORT, MOOSE, PRISMA, PRISMA for harms, STARD, STROBE, CHEERS, SQUIRE 2.0, or CHERRIES guidelines, as appropriate.

5. 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 and gynecology data definitions at https://www.acog.org/About-ACOG/ACOG-Departments/Patient-Safety-and-Quality-Improvement/reVITALize. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

6. 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 22 typed, double-spaced pages (5,500 words). Stated page limits include all numbered pages in a manuscript (i.e., title page, précis, abstract, text, references, tables, boxes, figure legends, and print appendixes) but exclude references.

7. Specific rules govern the use of acknowledgments in the journal. Please note the following guidelines:

* 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).

8. The most common deficiency in revised manuscripts involves the abstract. Be 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 paper. Make sure that the abstract does not contain information that does not appear in the body text. If you submit a revision, please check the abstract carefully.

In addition, the abstract length should follow journal guidelines. The word limits for different article types are as follows: Original Research articles, 300 words. Please provide a word count.

9. 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.

10. The journal does not use the virgule symbol (/) in sentences with words. Please rephrase your text to avoid using "and/or," or similar constructions throughout the text. You may retain this symbol if you are using it to express data or a measurement.

11. 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%").

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

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Please note that 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.

14. If you choose to revise your manuscript, please submit your revision through Editorial Manager at http://ong.editorialmanager.com. Your manuscript should be uploaded in a word processing format such as Microsoft Word. Your revision's cover letter should include the following:
* A confirmation that you have read the Instructions for Authors (http://edmgr.ovid.com/ong/accounts/authors.pdf), and
* A point-by-point response to each of the received comments in this letter.

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 Mar 26, 2020, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Nancy C. Chescheir, MD
Editor-in-Chief

2018 IMPACT FACTOR: 4.965
2018 IMPACT FACTOR RANKING: 7th out of 83 ob/gyn journals

RE: Manuscript Number ONG-20-262

Determinants of serum estradiol levels among postmenopausal women using hormone therapy

Dear Dr. Sriprasert:

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).

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REVIEWER COMMENTS:

Reviewer #1:

The authors of this manuscript present a post hoc analysis of the ELITE trial, a single-center, randomized, double blinded, placebo controlled trial of HT in healthy postmenopausal women stratified to early or late menopause. The stated objective of the study was to explore determinants of estradiol (E2) levels among study participants.

1. The citation describing the utility of measuring serum estradiol levels (Yasui, et al, 2001) was a small study evaluating different estradiol levels for different clinical endpoints. This is a small study with 34 patients in each of two arms receiving different doses of HT than used in this study. Measuring serum E2 levels is unfamiliar to most obstetrician gynecologists prescribing hormone therapy in the postmenopause as they do so for menopausal symptoms and titrate based upon symptoms. A more robust description of the findings of the authors previous work associating E2 levels and atherosclerosis would be more appropriate for the readership of this journal. Specific E2 levels are not currently guideline recommended within clinical care. When measured serum E2 levels 40-100 pg/mL are considered a reasonable target - is this consistent with the authors recommendations?

RESPONSE:

We appreciate the reviewer's concern regarding the size of the cited study and have removed the reference to this study.

We are not making any clinical recommendations based on serum E2 levels. Recommendations would have to be based on specific end point targeted, individual responsiveness to dosage (including absorption, catabolism of E2, excretion rate, genetics) and this would take many studies across many populations.

Certainly the single study we cited involving a small sample of women with limited end points could not provide such recommendations. We agree with the generally accepted targets of 40-100 pg/ml E2 level after taking HT.

There is unfortunately very little information regarding the association between E2 levels and different clinical outcomes in the literature. In addition, use of different E2 measurement assays may yield different E2 levels; for example, direct radioimmunoassay may overestimate while mass-spectrometry may underestimate E2 levels. Major differences in inter-subject variation may result in different E2 levels across studies simply due to use of different assays.

We revised our statement in the introduction to demonstrate the association between E2 levels and some biological effects among women taking HT. We now refer to one study showing different E2 levels by severity of hot flashes and a second study reporting higher serum E2 level with increased bone mass density.

"Some studies show that achieved E2 levels with HT treatment relate to biological effects of HT treatmentswan. For instance, in the Kronos Early Estrogen Prevention Study (KEEPS), among women randomized to transdermal E2, those women reporting no hot flashes had a geometric mean E2 of 44 pg/ml, which was significantly higher than women reporting moderate (9 pg/ml) or severe (11 pg/ml) hot flashes. In the Ultra-Low-dose Transdermal estRogen Assessment (ULTRA) trial, mean serum E2 levels increased from 4.5 to 8.6 pg/ml over the 2 years of the study. This resulted in a 2.6% mean increase in lumbar spine bone mineral density (BMD) and 1.2% increase in total hip BMD relative to placebo, along with significant decreases in serum levels of bone turnover markers."

In terms of association between E2 and atherosclerosis, we added a new reference of a study showing association between E2 dose and decreased atherosclerosis. We have also expanded the text describing our previous work from ELITE regarding the association between E2 level and atherosclerosis progression among early and late postmenopausal women. We added an additional reference showing consistent results of association between E2 level and atherosclerosis progression among postmenopausal women.

In response, we have added the following references;
Ref: Santoro N, Allshouse A, Neal-Perry G, Pal L, Lobo RA, Naftolin F, et al. Longitudinal changes in menopausal symptoms comparing women randomized to low-dose oral conjugated estrogens or transdermal estradiol plus micronized progesterone versus placebo: the Kronos Early Estrogen Prevention Study. Menopause 2017 Mar;24(3):238-46.

Ref: Ettinger B, Ensrud KE, Wallace R, Johnson KC, Cummings SR, Yankov V, et al. Effects of ultralow-dose transdermal estradiol on bone mineral density: a randomized clinical trial. Obstet Gynecol 2004 Sep;104(3):443-51.

Ref: Ostberg JE, Storry C, Donald AE, Attar MJ, Halcox JP, Conway GS. A dose-response study of hormone replacement in young hypogonadal women: effects on intima media thickness and metabolism. Clin Endocrinol (Oxf) 2007 Apr;66(4):557-64.

Ref: Karim R, Hodis HN, Stanczyk FZ, Lobo RA, Mack WJ. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. J Clin Endocrinol Metab 2008 Jan;93(1):131-8.

2. Are there harms associated with higher serum E2 levels in either the later or early menopausal patient population. Since E2 levels are infrequently drawn in clinical practice these are not well described in the literature but are relevant to the topic of this manuscript.
RESPONSE:
According to our prior study on the association between E2 level and atherosclerosis progression, higher E2 levels were significantly associated with greater atherosclerosis progression among late postmenopausal women. We have expanded our description of these findings in the revised manuscript.

Ref: Sriprasert I, Hodis HN, Karim R, Stanczyk FZ, Shoupe D, Henderson VW, et al. Differential effect of plasma estradiol on subclinical atherosclerosis progression in early versus late postmenopause. J Clin Endocrinol Metab 2018 Sep 28.

3. Another issue with serum estradiol levels is levels peak and trough during the day. Were estradiol levels checked at the same time of day? Was this variability accounted for in any of the results? Across trial variability was not described. Did patients E2 levels change during the time they were followed on study?
RESPONSE:
We measured the estradiol level from fasting blood samples collected in the morning during all baseline and follow-up visits. There was significant within-subject variability in E2 levels across follow-up visits (p=0.01). We statistically accounted for this within-subject variability in the mixed effects linear model by including a random participant-level intercept to model variability around population-level means.

4. The discussion nicely summarizes the factors which influence E2 levels - BMI, Alcohol, Smoking, Antifungal medicine, but does not summarize how these findings are important to a prescriber. Should doses be modified based upon these factor? 1mg daily of estradiol is a dose higher than is typically used in postmenopausal HT - do these factors warrant dose modifications for women using MHT?
RESPONSE:
Results from this study were from only 1 mg oral estradiol. Despite the fact that we did not study other doses, we may assume that these determinants also affect achieved estradiol levels using different doses of estradiol. Therefore, it may be worthwhile for physicians to consider these identified factors (BMI, alcohol and smoking) relative to a woman's menopausal symptoms to adjust estradiol dose.

5. In Tables 1 and 2, Hispanic is listed as a category for race. Recommend using census terms andethnicity. https://urldefense.com/v3/__https://www.census.gov/mso/www/training/pdf/race-ethnicity-onepager.pdf__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJaRHXq4eQ$
RESPONSE: We have revised the race/ethnicity variable in Tables 1 and 2 according to the census terms.

6. In Table 2, would recommend including the E2 values for each group.
RESPONSE:
We added median estradiol level for each categorical variable in Table 2. We also report the estimated least square mean estradiol level for each determinant included in the final model in Supplementary Table 3.

Reviewer #2:

Precis - BMI, surgical menopause, alcohol use, smoking, and antifungal medications are determinants of serum estradiol levels among postmenopausal women on HRT

Abstract - Objective: benefit of HT - atherosclerosis related to E2 levels - identify determinants of E2 on healthy women on HT
Methods - women from ELITE trial RCT - 1 mg E2 +/- vaginal progesterone with 80% compliance; E2 measured q 6 mo over median 4.8 yrs; demographics, clinical characteristics, medication use, biomarkers of liver/kidney function all collected
Results - 275 women - avg age / time to menopause in early vs late menopause - 55.4 vs 64.4 yr and 3.6 versus 16 years
adjusted for pretreatment E2 - increased E2 if higher BMI, higher creatinine, surgical menopause, etoh use and antihypertensive medications; smoking and antifungal use was associated with lower E2
multivariable model - increased BMI, surgical menopause, and alcohol associated with higher E2 and smoking and antifungals were associated with lower E2; the determinants were the same for early versus late menopause
Conclusions - determinants for E2 - BMI, surgical menopause, etoh, smoking, antifungal medications; E2 relates to treatment efficacy and most HT determinants are modifiable; the desirable E2 levels can be obtained through personal intervention

Introduction - E2 levels are different for different treatment endpoints - reviewed; HT has decreased risk coronary heart disease/atherosclerosis in early menopause but no affect in late menopause; ELITE trial findings - decrease atherosclerosis with E2 in early menopause but increased in late; goal is to explore determinants of E2 levels

Methods - study design - post-hoc analysis of ELITE RCT - < 6 yr after menopause vs > 10 yr; 1 mg E2 +/- vaginal progesterone - followed between 1/05-2/13 for avg 4.8 yr
primary results - HT - decreases progression of atherosclerosis when initiated early but not late
80% compliance by tablet count to be evaluated; serum E2 measurements at baseline and q 6 months
Measurement E2 correlates - race, type of menopause, vitamins/ fish oil, weight, BMI, smoking, Etoh, exercise, medications, kidney and liver function - assessed q 6-12 months
Statistical analysis - described

Results - 275 women with adequate compliance - 123 with early menopause and 152 with late
baseline characteristics - age 55 vs 64 time since menopause 3.6 yr vs 16 yr
Serum E2 - baseline - early and late was the same - approx. 10; on trial E2 mean and median was the same between early and late menopause - approx 52-56
association between log transformed E2 and potential correlates - increased E2 associated with BMI, surgical menopause, Etho and antihypertensive medications; decreased E2 associated with smoking and antifungal meds
past smokers had lower E2 and current smokers had the lowest E2; Etoh had increased E2 -

other factors were not associated
Multivariable association log transformed E2 - elevated E2 associated with BMI and etoh use
surgical menopause - increased E2 borderline statistically significant
lower E2 with smoking and antifungals
determinants of E2 similar between early and late menopause

Discussion - BMI, surgical menopause, ETOH use, smoking, and antifungal medications had statistically significant correlation with E2 levels - same between early and late menopause
the association with alcohol is stronger with early menopause and biological explanation involves E2 metabolism
BMI - increased BMI leads to increased E2 through peripheral aromatization
ETOH - biological rationale reviewed
smoking - increased hepatic metabolism with oral E2 but not transdermal and decreased benefit of symptoms with smokers
antifungal meds -impact on metabolism
nonsignificant variables - other medications and exercise
Strengths/ limitations - RCT data, determinants of E2 level in women with good compliance but measures total E2 and not other metabolites
surgical menopause - no explanation and small group - 11%

Public health/ conclusions - benefits of HT on atherosclerosis related to E2 levels and significant determinants of E2 - weight, etoh, smoking - are modifiable
consider control of weight, smoking, etoh in achieving lowest effective dose with minimum side effects for each patient

Comments -
This is overall a well-written good analysis of statistics and important to see the effect of various factors on estradiol levels with HT.

1. Statistics should be reviewed by a statistician
RESPONSE:
The manuscript review included a statistical reviewer.

2. Not sure there are enough patients with surgical menopause to list this as a criteria given that there are few patients and data is only borderline, and there is no real biological plausibility
RESPONSE:
Because of the small number of surgically menopausal participants and the findings of borderline statistical significance (p=0.54 in the multivariable model), we removed surgical menopause from the final model.

3. Perhaps mention of threshold of alcohol use should be mentioned as it appears that it is only relevant in women with use > 2 drinks per day so mild alcohol use was not a determinant
RESPONSE:

We now point out the amount of alcohol of >2 drinks per day as a significant determinant of serum E2 level. We choose to retain the discussion of the trend in E2 levels with level of alcohol use.

“There was a significant overall association between level of alcohol consumption and serum E2 ($p<0.001$). Alcohol use of more than 2 drinks per day was significantly associated with higher serum E2 level ($p<0.001$). The beta coefficients showed a trend in increasing serum E2 level with the amount of alcohol use (p trend=0.002).”

Reviewer #3:

1. The statement "E2 levels are associated with HT treatment efficacy" (line 79 and again line 318) is vague and not supported with a reference. This statement, which is the crux of the author's conclusion, is heavily dependent on how the author defines "efficacy." Is efficacy control of vasomotor symptoms? Reducing risk of osteoporosis-related fractures? Alteration of lipid profile?

Trying to figure out what "efficacy" means in the context of this research, I was unable to find evidence that ties E2 level to patient perception of vasomotor symptom relief. Yasui (2001), which you cited, actually states that the Kupperman index (a questionnaire of vasomotor symptoms) is not related to serum estradiol level. Bone loss does appear to be reduced in HT users and there appears to be conflicting information about carotid intima-media thickening in young HT users. And while some markers of lipid profile like HDL and LDL do improve on oral estrogen, others like triglycerides do not, so I don't think this is what is meant by "efficacy" in the context of this research.

Since your conclusion states that important public health implications stem from your statement that "achieved E2 levels relate to the HT treatment efficacy," I think a more specific description of what you mean by "efficacy" is vital. Otherwise, the concept of efficacy is left up to the reader and they may draw a conclusion (related to vasomotor symptom relief, for example) that is not actually supported by your research. Something along the lines of "achieved E2 levels relate to the HT treatment biological effect" rather than efficacy seems more accurate and less confusing.

RESPONSE:

Thank you so much for pointing out this issue with our statement. We agree that we cannot infer a general statement regarding efficacy from the results of a small single study; we removed the Yasui (2001) study from our manuscript.

We further revised our text regarding the association between E2 levels and biological effects among women taking HT. We have added references to one study showing different E2 levels with severity of hot flashes in women treated with transdermal E2, and another study reporting the increased serum E2 level along with increased bone mass density in women treated with ultra-low dose transdermal E2.

“Some studies show that achieved E2 levels with HT treatment relate to biological effects of HT treatment. For instance, in the Kronos Early Estrogen Prevention Study (KEEPS), among women randomized to transdermal E2, those women reporting no hot flashes had a geometric mean E2 of 44 pg/ml, which was significantly higher than women reporting moderate (9 pg/ml) or severe (11 pg/ml) hot flashes. In the Ultra-Low-dose Transdermal estRogen Assessment (ULTRA) trial, mean serum E2 levels increased from 4.5 to 8.6 pg/ml over the 2 years of the study. This resulted in a 2.6% mean increase in lumbar spine bone mineral density (BMD) and 1.2% increase in total hip BMD relative to placebo, along with significant decreases in serum levels of bone turnover markers.”

In terms of association between E2 and atherosclerosis, we added a new reference of a study showing association between E2 dose and decreased atherosclerosis. We have also expanded the text describing our previous work from ELITE regarding the association between E2 level and atherosclerosis progression among early and late postmenopausal women. We added an additional reference showing consistent results of association between E2 level and atherosclerosis progression among postmenopausal women.

In response, we have added the following references;
Ref: Santoro N, Allshouse A, Neal-Perry G, Pal L, Lobo RA, Naftolin F, et al. Longitudinal changes in menopausal symptoms comparing women randomized to low-dose oral conjugated estrogens or transdermal estradiol plus micronized progesterone versus placebo: the Kronos Early Estrogen Prevention Study. Menopause 2017 Mar;24(3):238-46.

Ref: Ettinger B, Ensrud KE, Wallace R, Johnson KC, Cummings SR, Yankov V, et al. Effects of ultralow-dose transdermal estradiol on bone mineral density: a randomized clinical trial. Obstet Gynecol 2004 Sep;104(3):443-51.

Ref: Ostberg JE, Storry C, Donald AE, Attar MJ, Halcox JP, Conway GS. A dose-response study of hormone replacement in young hypogonadal women: effects on intima media thickness and metabolism. Clin Endocrinol (Oxf) 2007 Apr;66(4):557-64.

Ref: Karim R, Hodis HN, Stanczyk FZ, Lobo RA, Mack WJ. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. J Clin Endocrinol Metab 2008 Jan;93(1):131-8.

2. You referenced Ginsburg JAMA 1996 which described the relationship between alcohol consumption and serum estradiol level. Your research provides a much larger sample size which supports that earlier finding. So while this finding is not new, it is potentially helpful to see this finding supported by a larger sample size.
RESPONSE:
We thank the reviewer for this observation regarding validation of the alcohol association with a larger sample.

3. In the paragraph "Associations between log-transformed on-trial E2 levels and potential correlates" (line 196) that follows with the statement "Adjusted for baseline serum E2 level..." you mention that creatinine is "not significantly associated with serum E2 levels." However, in the results section of your abstract, you list higher creatinine being associated with higher serum E2 levels (line 54). This appears to be conflicting information. I don't see anywhere in the rest of the article documenting any association with E2 level and creatinine. If it is a discrepancy, it should be corrected. If it is not a discrepancy, it might be worthwhile clarifying in the body of the article exactly how a positive relationship between E2 and creatinine WAS found. It seems confusing to me and clarifying it might help me and other readers.
RESPONSE:

We apologize for the error and have corrected the abstract by removing the statement about creatinine.

4. Overall, the article further supports the idea that improving modifiable lifestyle factors such as weight, smoking and alcohol use are important (which is already supported by ample other evidence in the literature). While these recommendations are not novel, being able to better educate patients that are taking or considering HT is likely helpful.
RESPONSE:
Thank you so much for the supporting statement regarding the significance of this work.

Reviewer #4:

This is a well-written manuscript with succinct text and excellent display of the data. The topic is important and has implications for clinical practice. I have a few comments.

1. The authors confidently state that serum E2 levels < 15, >=15 and >= 25 pg/ml are associated with different tissue effects, but this is based upon a single 2001 reference (Reference 3) with only 25 women in the < 15 group, 27 women in the >=15 to < 25 group and 16 women in the >=25 group. That article suggests in the discussion that low concentrations of estradiol (< 15 pg/ml) was sufficient to suppress LH and FSH "and to relieve VMS", but the article shows no results for VMS. Please provide reference for estradiol and decrease in VMS and additional references to support thresholds for symptom and tissue effects of serum estradiol.
RESPONSE:
We agree with the reviewer that we should not draw a strong conclusion from a small study; We therefore removed that study from our manuscript.

We further revised our text regarding the association between E2 levels and biological effects among women taking HT. We have added references to one study showing different E2 levels with severity of hot flashes in women treated with transdermal E2, and another study reporting the increased serum E2 level along with increased bone mass density in women treated with ultra-low dose transdermal E2.

"Some studies show that achieved E2 levels with HT treatment relate to biological effects of HT treatment. For instance, in the Kronos Early Estrogen Prevention Study (KEEPS), among women randomized to transdermal E2, those women reporting no hot flashes had a geometric mean E2 of 44 pg/ml, which was significantly higher than women reporting moderate (9 pg/ml) or severe (11 pg/ml) hot flashes. In the Ultra-Low-dose Transdermal estRogen Assessment (ULTRA) trial, mean serum E2 levels increased from 4.5 to 8.6 pg/ml over the 2 years of the study. This resulted in a 2.6% mean increase in lumbar spine bone mineral density (BMD) and 1.2% increase in total hip BMD relative to placebo, along with significant decreases in serum levels of bone turnover markers."

2. The authors state mean serum E2 level achieved was 46.5 (30.6) pg/ml, range 5 to 330 pg/ml in their trial population (5). Please comment on this wide range in this select population of adherent women taking E2. Or is this among adherent and nonadherent women and if so, only provide range for adherent women. If this is for adherent women, why is the range so wide?
RESPONSE:
We revised the range to represent E2 levels measured when women were at least 80% adherent by pill count during the follow-up visits. The range was 9.9-260.3 pg/ml. Indeed, this wide range of estradiol levels among adherent women using hormone therapy intrigued us, and led us to hypothesize that there could be potential determinants of estradiol level apart from compliance. The marked variability was the basis for our objective of this study to explore other factors influencing estradiol levels.

3. Please comment on when E2 serum samples were obtained in relation to the 4% vaginal micronized progesterone administration and why or why not this might matter.
RESPONSE:
We did not record the timing of the vaginal micronized progesterone application in relation to the E2 samples.

In response to this reviewer's query, we performed two additional analyses:
(1) We correlated E2 levels with serum P4 levels measured at the same time.
Serum progesterone was not correlated with serum estradiol level (correlation coefficient = -0.01, p=0.67).

(2) We compared E2 levels in women who did and did not use vaginal progesterone (i.e. with and without a uterus).
Women with a uterus (who use progesterone) had a median (IQR) E2 of 43 (37) pg/ml and women without a uterus (who did not use progesterone) had a median (IQR) E2 of 57 (44) pg/ml. The medians were not statistically different between two groups of women (p=0.35).

From additional analyses of our data, we conclude that progesterone level does not significantly affect the estradiol levels. Therefore, we concluded that the 4% vaginal micronized progesterone administration did not contribute to the serum estradiol levels in the analysis.

In addition, despite an adequate endometrial effect, vaginal progesterone has been reported to result in levels in the systemic circulation that are 10-fold less than systemic progesterone administration (Ref: Warren MP. Vaginal progesterone and the vaginal first-pass effect. Climacteric 2018 Aug;21(4):355-7.). Although progesterone can be converted into E2 via the steroid pathway, with a minimal level of progesterone in the circulation from vaginal administration, we would not expect it to contribute to E2 levels studied here. Indeed, our observed correlation of -0.01 between serum levels of progesterone and E2 support this conclusion.

4. Methods, In your tables and text, did I miss the list the covariates in the models that were included?
RESPONSE:
All potential E2 correlates tested are listed in the methods section (sub-section header; Measurement of potential E2 correlates). In Table 2, we tested each potential correlate separately. Table 3 reported the significant correlates in the final multivariable model.

5. How did you account for repeated measures, i.e. you had 2160 E2 measures in 275 women.
RESPONSE:
We used a mixed effects linear model with random intercept for each participant to model the fact that participants provided repeated observations.

6. I was surprised by similar baseline E2 levels between early and late menopause participants.

How do you explain this?
RESPONSE:
The reviewer makes a good point. We are not clear on the reason for the similar E2 levels. The similar baseline E2 levels may have to do with the trial selection criteria, including eligibility criteria among participants with estradiol level of <25 pg/ml as an indicator of postmenopausal status. In addition, selection of a healthy population of women without CVD and diabetes, since both of these disease processes are driven by E2 deficiency in women, may have contributed to the similar baseline E2 levels.

In support of our findings, previous studies have shown that age was not associated with serum E2 levels among postmenopausal women not taking HT, whereas SHBG concentration increased significantly with age.
(Ref: Laughlin GA, Barrett-Connor E, Kritz-Silverstein D, von Muhlen D. Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo Study. J Clin Endocrinol Metab 2000 Feb;85(2):645-51.
Ref: Karim R, Mack WJ, Hodis HN, Roy S, Stanczyk FZ. Influence of age and obesity on serum estradiol, estrone, and sex hormone binding globulin concentrations following oral estrogen administration in postmenopausal women. J Clin Endocrinol Metab 2009 Nov;94(11):4136-43.)

Therefore, although early and late postmenopausal women in our study had similar total E2 level, it is possible that free E2 levels in late postmenopausal women would be lower than early postmenopausal women due to higher SHBG levels. As the current study was limited to serum total E2 levels, further data on SHBG and free E2 levels are needed to possibly explain this finding.

7. Your explanation for higher E2 levels among surgically menopause women, "a unique population sampling" is a single explanation of your findings, but please remind the reader only 30 women had surgical menopause with? 15 women each in the early and late postmenopausal groups? More likely explanation of these findings is existing confounding that is not accounted for, like obesity or alcoholism or other. Please comment.
RESPONSE:
Please note that the analysis used 2160 repeated E2 measurements (1916 natural vs 244 surgical menopause) from this sample of 275 women (245 natural vs 30 surgical menopause). We reran the analysis and surgical menopause was removed from the final model due to its marginal significance (p=0.054) in the multivariable model. In the discussion of the unadjusted results, we note the small sample

8. Why would past smoking affect serum E2 levels, and in addition, why was this observed only in your younger women (early postmenopausal)?
RESPONSE:
We were intrigued by this result also. As such, we explored this question further in subsequent data analysis.

First, we hypothesized that cessation of smoking may be reported in women who had recently stopped smoking. Analysis on time since quitting smoking among the past smokers showed a median quit time of 28 years. The correlation between time since stopped smoking was weakly inversely correlated with serum E2 level (correlation coefficient -0.19 p=0.06). Only 9 women (representing 27 E2-measured visits) had recently stopped smoking during the trial. After removing these 27 visits from the analysis, the results did not change (beta (SE) -0.1496 (0.0567) p=0.008 for past smoking).

Secondly, we hypothesized that past smokers may have been exposed to secondhand smoking. We found that 6 past smokers (representing 9 E2-measured visits) were exposed to secondhand smoking in their homes, 8 smokers (representing 14 E2-measured visits) were exposed to secondhand smoking outside of their home, and 12 smokers (representing 22 E2-measured visits) were exposed to secondhand smoking in places outside of home and work. After excluding these participants with secondhand smoking exposure from the analysis, the results did not change (beta (SE) -0.1664 (0.0554) p=0.003 for past smoking).

In summary, the effect of past smoking on serum estradiol level does not appear to be due to recent smoking cessation or to secondhand smoking.

We were disappointed that our data did not reveal a precise answer to the question of past smoking and its association with E2 levels. One thought we have is that the induction of liver enzymatic activity by smoking may be permanent or may take many decades to return to baseline levels, similar to the decreasing risk over decades of lung cancer seen in individuals who stop smoking. However, this is conjecture and will require further study to untangle this interesting but complex finding.

9. Only 2 women used antifungals at baseline, yet there are 45 samples in which women were using antifungals, is this correct? Please discuss as limitation.
RESPONSE:
There were 2 women using antifungal medication at baseline. However, 18 women contributed 45 visits in which antifungal medications were used during the on-trial follow-up; these 45 visits represent the analysis of antifungal medication use in relation to E2 levels. We have removed antifungal medication use from the multivariable model, for more robustness as it has limited sample size.

10. Line 312, there is an extra "the".
RESPONSE:
We have revised the conclusion regarding surgical menopause since it was removed from the multivariable model.

Surgical menopause was significantly associated with higher E2 levels compared with natural menopause, however, it was at borderline statistical significance (p=0.054) in multivariable model, therefore, it was dropped from the final model.

11. Can you comment in discussion about serum E2 and race (SWAN references)?
RESPONSE:
We addressed the SWAN references on E2 level across race/ethnicity in the manuscript as follows;

"Endogenous serum E2 levels among premenopausal and perimenopausal women significantly differed across race/ethnicity at menopausal transition in the Study of Women Across the Nation (SWAN), with highest serum E2 levels in Hispanic women and lowest levels in Asian women. The difference in serum E2 level disappeared after adjustment for BMI (Ref: Randolph JF, Jr., Sowers M, Gold EB, Mohr BA, Luborsky J, Santoro N, et al. Reproductive hormones in the early menopausal transition: relationship to ethnicity, body size, and menopausal status. J Clin Endocrinol Metab 2003 Apr;88(4):1516-22.). In ELITE participants who were postmenopausal women taking HT, race/ethnicity were not related to achieved serum E2 levels while BMI was significantly associated with serum E2 level. These findings may suggest that metabolism of both endogenous and exogenous E2 is related to BMI rather than race/ethnicity."

We also evaluated the endogenous E2 level at baseline in our data and did not find significantly different levels by race and ethnicity. The mean and median baseline E2 levels before HT treatment in our study population are listed below.

	N	Mean (SD)	Median (IQR)	Min, Max
Race/Ethnicity				
White, non-Hispanic	202	10.9 (3.4)	9.0 (3.0)	9.0, 25.0
African American, non-Hispanic	21	11.2 (3.2)	9.0 (5.0)	9.0, 18.0
Hispanic	30	10.2 (2.6)	9.0 (2.0)	9.0, 19.0
Asian, non-Hispanic	22	10.0 (2.5)	9.0 (0)	9.0 (17.0)

12. Only 9 women had > 2 drinks per day, what was the breakdown by early and late menopause? Please discuss as limitation.
RESPONSE:
This number is the sample size at baseline. In the analysis, 11 women contributed 42 visits with >2 drinks per day to the analysis of on-trial estradiol levels. We note this relatively smaller sample size as a limitation in the discussion section.

13. Discussion on ETOH - you show differences in mean E2 levels between individuals with no ETOH, < 1 ETOH and 1-2 drinks. Would it be fair to more explicitly recommended less than <1 drink per day in your conclusions until a study can be done with more numbers? Please comment.
RESPONSE:
We stated the limitation regarding alcohol use as "Despite the overall significant trend in serum E2 levels by level of daily alcohol use, the estimate of association with alcohol intake of more than 2 drinks per day was based on a small sample size, thus needs further exploration with a

larger sample size." We also revised our public health implication specifically on 2 or more drinks of alcohol.

14. Your conclusion, "Postmenopausal women should control their weight and refrain from smoking and alcohol use with the goal of obtaining the lowest effective dose of HT," is fair but rather vague. More explicitly, should you suggest, women who are obese and cannot lose weight and who drink more than 1 drink per day who are 10 years from menopause may be at higher risk for atherosclerosis due to higher estrogen levels or if taking HT, lower doses may be sufficient for optimal tissue effects. And, women who cannot stop smoking may need more estrogen to attain adequate serum estrogen levels and to decrease fracture risk.
RESPONSE:
We have added the detailed conclusion in the manuscript as suggested.

Postmenopausal women who are obese, drink more than 2 alcoholic beverages per day and are 10 years since menopause may be at higher risk for atherosclerosis due to higher E2 levels when taking HT. Postmenopausal women who continue to smoke may need higher E2 dosages to attain serum E2 levels adequate to decrease fracture risk.

15. Not all would agree with your statement, "In particular, we have previously reported that the beneficial effect of HT on atherosclerosis progression among postmenopausal women is related to achieved serum E2 levels.(6)" Please temper this statement by referencing studies that do not agree with your prior findings, or qualify.
RESPONSE:
We revised our statement as "This study has important public health implications for postmenopausal women taking HT, as achieved E2 levels relate to HT biological response. In particular, we have previously reported that the effect of HT on atherosclerosis progression among postmenopausal women is related to achieved serum E2 levels. Higher E2 levels were associated with reduced atherosclerosis progression when initiated in early postmenopause (<6 years since menopause); however, higher E2 levels were associated with greater atherosclerosis progression when initiated in late postmenopause (≥10 years since menopause).

STATISTICAL EDITOR COMMENTS:

The Statistical Editor makes the following points that need to be addressed:

1. Table 2: The "n" for subsets (eg, smoking status: never, past or current, alcohol use, etc) should include the number of women in those categories, in addition to the number of measurements.
RESPONSE:
We added number of women in additional to the number of measurements for each variable in the table as suggested.

2. There are multiple comparisons in this Table, with no adjustment for multiple comparisons. Some of the comparisons are likely spurious (eg, surgical vs natural menopause, highest tertile of weekly hrs of physical activity, use of antihypertensives or antifungals (additional issue is the sample of only 2 women for antifungal use, insufficient to infer general conclusions re: E2 levels from these data) and smoking status or alcohol use, which only achieve statistical significance by p-trend, by individual categories, were either NS or marginally so.
RESPONSE:
Results in Table 2 showed the analysis for each determinant separately adjusted for baseline estradiol level and the reduced estradiol dose variable.
We revised our interpretation for the trend test and categorical variable only for the categories that showed statistical significance.
Despite the 2 women using antifungal medication at baseline, in the analysis, we included 45 visits from 18 women with antifungal medication use during the trial.

3. Also, the formatting of ß and their SE by log, rather than by transformed values, is not very useful for clinicians. Should include the log values as on-line supplemental, and use transformed values in main text.
RESPONSE:
We revised the beta and SE by log into the transformed values and 95% confidence interval. We moved the beta and SE by log to the Supplementary Table1.

4. Table 3: Need to include the "n" for each subset (number of women and number of measurements) when stratified by early vs late menopause.
RESPONSE:
We added number of women in additional to the number of measurements for each variable in the table as suggested.

5. Since the antifungal use had N = 2 women, either there was one woman in each category of early vs late or the values were derived by the model, inferring the effect of antifungal use in each stratum. In any case, the number of women using antifungals is insufficient to include in a general model of E2 levels. Should omit from the model.
RESPONSE:

Despite the 2 women using antifungal medication at baseline, in the analysis, we included 45 visits from 18 women with antifungal medication use.

We removed antifungal medication form the final model as suggested for a more robust final model.

6. Also, although the overall p-value trends for E2 level vs alcohol use were significant for the total and for the early menopause groups, when compared categorically, only the highest intake level was actually significantly associated with higher E2 levels. Therefore, the general conclusion that E2 is associated with alcohol intake should be clarified, since it only applied to analysis of the highest intake group. That group had n = 9 women with only 45 measurements and the reader cannot tell how many of those were in the early vs late stratum. Extrapolating from those relatively sparse data to a general conclusion about alcohol intake vs E2 seems imprecise. In summary, the conclusion from Table 3 is an association of E2 with BMI, but not surgical vs natural menopause, a negative association between E2 and smoking status, but only for the early menopause stratum and associations of E2 vs the highest level of alcohol use or the use of antifungals, which were based on small samples and may not be reproducible.
RESPONSE:
We revised the interpretation of Table 3 as suggested.

7. lines 63, 164-170, 214-215: Borderline is actually NS by the threshold cited.
RESPONSE:
We have removed this variable from the final model.

8. lines 262-278 and Tables 2, 3: How do these mechanisms account for the association of E2 levels vs past smokers?
RESPONSE:
Please refer to our response to reviewer #4 item 8

EDITOR'S COMMENTS

We no longer require that authors adhere to the Green Journal format with the first submission of their papers. However, any revisions must do so. I strongly encourage you to read the instructions for authors (the general bits as well as those specific to the feature-type you are submitting). The instructions provide guidance regarding formatting, word and reference limits, authorship issues, and other things. Adherence to these requirements with your revision will avoid delays during the revision process, as well as avoid re-revisions on your part in order to comply with the formatting. For instance, we don't use headings such as "Study design" in the different paper sections.

1. Line 33: "Determine" seems like too strong a causal word here. Based on your data, it seems that "influence" may be better. None of these are deterministic. Similar wording, used elsewhere such as line 62 and line 64, should be edited. Rather than Determinants, perhaps "factors" or something similar.
RESPONSE: We revised the wording as suggested throughout the manuscript.

2. In the abstract, please provide the standard deviations.
RESPONSE: We added standard deviations as suggested.

3. PRESENTATION OF STATS INFORMATION
P Values vs Effect Size and Confidence Intervals: While P values are a central part of inference testing in statistics, when cited alone, often the strength of the conclusion can be misunderstood. Whenever possible, 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.

This is true for the abstract as well as the manuscript, tables and figures.

Please provide absolute values for variables, in addition to assessment of statistical significance.
RESPONSE:
We reported the back-transformed beta coefficient with 95% confidence interval along with the p-value in Table 2 and Table 3.

The untransformed beta coefficient with SE are now presented in Supplementary Table 1 and 2.

We added observed median E2 levels for all categorical variables in Table 2

We present predicted mean E2 levels with 95% confidence interval from the final model in supplementary Table 3.

4. We ask that you provide crude OR's followed by adjusted OR's for all relevant variables.
RESPONSE:
We used linear mixed effects regression model in the analysis on the continuous outcome of E2 level; the results shown are beta coefficients as estimates of effect size. We assume that the editor is requesting presentation of unadjusted and adjusted beta coefficients (not ORs). Our minimally adjusted estimates are provided in Table 2, and adjusted estimates in Table 3. Given the stratification by early and late postmenopause, presentation of unadjusted and adjusted estimates in a single table would be very unwieldy. We will however adhere to any further editorial guidance on this.

5. The objective of the abstract should be a simple "To" statement without background information.
RESPONSE:
We revised the objective in the abstract as suggested.

6. Line 71: Maybe used "by" postmenopausal women?
RESPONSE:
We revised as suggested.

7. Line 92: in the 2018 NAMS statement about hormone therapy, the recommendation is to individual treatment formulation, dose and route to achieve desired end but from my review of this document, there is no recommendation to follow serum levels in this management recommendation. It seems appropriate for you to at least acknowledge this either here or in the discussion section, preferably in the introduction in order to put this into context for the reader. It seems prudent to make it clear that you are not recommending following serum E2 levels.
RESPONSE:
We revised the statement as follows;
The North American Menopause Society in 2017 recommended that HT should be individualized to maximize benefits and minimize risks. Although there is no recommendation to monitor serum E2 levels among postmenopausal women taking HT, achieved E2 levels have been shown to be associated with potential benefits on atherosclerosis. Therefore, it is important to identify factors related to achievement of adequate E2 levels.

8. Line 96: again, determinants.
RESPONSE:
We revised the wording as factors associated with serum E2 level.

9. Line 102: was this a planned post hoc analysis? I really appreciate your clear description of the original trial and the outcomes.
RESPONSE:
This was not a planned post-hoc analysis. We have revised the trial description to indicate this.

10. Line 199 Please limit p values to 3 decimal places.
RESPONSE:
We revised all p values to 3 decimal places throughout the manuscript.

11. Line 215: We do not allow authors to describe variables or outcomes in terms that imply a difference (such us of the terms “trend” or “tendency” or “marginally different”) unless there is a statistical difference. Please edit here and throughout to indicate that there is no difference.
RESPONSE:
We now limit outcome descriptions to those showing statistical difference.

12. Line 245: please avoid causal language here. This is an association. Please edit this language here and where ever it occurs in the report.
RESPONSE:
We revised the wording to avoid causal language as suggested.

13. Line 279 Please note statistical reviewer comments regarding caution with respect to antifungal data interpretation, given the very small number of exposed patients.
RESPONSE:
We have removed antifungal medication variable from the multivariable model, limiting the model to more robust variables.

14. Line 322: This is a rather narrow goal for recommending women control their weight, refrain from smoking and limit alcohol use. Perhaps achieving the lowest dose of HT could be one goal for these overall health goals but not THE goal?
RESPONSE:
We revised the statement as suggested.

EDITORIAL OFFICE COMMENTS:

1. The Editors of Obstetrics & Gynecology are seeking to increase 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:

A. OPT-IN: Yes, please publish my point-by-point response letter.

B. OPT-OUT: No, please do not publish my point-by-point response letter.

RESPONSE:

OPT-IN Yes, please publish my point-by-point response letter.

2. As of December 17, 2018, Obstetrics & Gynecology has implemented an "electronic Copyright Transfer Agreement" (eCTA) and will no longer be collecting author agreement forms. When you are ready to revise your manuscript, you will be prompted in Editorial Manager (EM) to click on "Revise Submission." Doing so will launch the resubmission process, and you will be walked through the various questions that comprise the eCTA. Each of your coauthors will receive an email from the system requesting that they review and electronically sign the eCTA.

Please check with your coauthors to confirm that the disclosures listed in their eCTA forms are correctly disclosed on the manuscript's title page.

RESPONSE:

We will comply with the requirement.

3. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript's lead author. The statement is as follows: "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." *The manuscript's guarantor.

If you are the lead author, please include this statement in your cover letter. If the lead author is a different person, please ask him/her to submit the signed transparency declaration to you. This document may be uploaded with your submission in Editorial Manager.

RESPONSE:

We added the statement in the revised cover letter as required.

4. The Editors believe this secondary analysis should follow STROBE guidelines. If you believe this is an incorrect assessment, please let us know which guideline you intend to follow in your revision's cover letter.

Responsible reporting of research studies, which includes a complete, transparent, accurate

and timely account of what was done and what was found during a research study, is an integral part of good research and publication practice and not an optional extra. Obstetrics & Gynecology supports initiatives aimed at improving the reporting of health research, and we ask authors to follow specific guidelines for reporting randomized controlled trials (ie, CONSORT), observational studies (ie, STROBE), meta-analyses and systematic reviews of randomized controlled trials (ie, PRISMA), harms in systematic reviews (ie, PRISMA for harms), studies of diagnostic accuracy (ie, STARD), meta-analyses and systematic reviews of observational studies (ie, MOOSE), economic evaluations of health interventions (ie, CHEERS), quality improvement in health care studies (ie, SQUIRE 2.0), and studies reporting results of Internet e-surveys (CHERRIES). Include the appropriate checklist for your manuscript type upon submission. Please write or insert the page numbers where each item appears in the margin of the checklist. Further information and links to the checklists are available at https://urldefense.com/v3/__http://ong.editorialmanager.com__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJa2iC4fSI$. In your cover letter, be sure to indicate that you have followed the CONSORT, MOOSE, PRISMA, PRISMA for harms, STARD, STROBE, CHEERS, SQUIRE 2.0, or CHERRIES guidelines, as appropriate.

RESPONSE:

We completed and attached the STROBE guidelines as requested with the resubmission.

5. Standard obstetrics 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 and gynecology data definitions at https://urldefense.com/v3/__https://www.acog.org/About-ACOG/ACOG-Departments/Patient-Safety-and-Quality-Improvement/reVITALize__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJaVhXngc$. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

RESPONSE:

We reviewed the definition of terms we used relative to the reVITALize definitions and complied with the recommendations.

6. 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 22 typed, double-spaced pages (5,500 words). Stated page limits include all numbered pages in a manuscript (i.e., title page, précis, abstract, text, references, tables, boxes, figure legends, and print appendixes) but exclude references.

RESPONSE:

The revised version of this manuscript contains a total of 5106 words (3377 words for the main text and 1729 words for tables and legends).

7. Specific rules govern the use of acknowledgments in the journal. Please note the following

guidelines:

* 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).
RESPONSE:
Financial support for this study is provided on the title page.
The authorship list reflects all contributors to the manuscript; there are no additional contributors. The presentation of this material at the NAMS meeting is noted on the title page.

8. The most common deficiency in revised manuscripts involves the abstract. Be 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 paper. Make sure that the abstract does not contain information that does not appear in the body text. If you submit a revision, please check the abstract carefully.

In addition, the abstract length should follow journal guidelines. The word limits for different article types are as follows: Original Research articles, 300 words. Please provide a word count.
RESPONSE:
We double checked that information in the revised version of the abstract is consistent with the information reported in the main manuscript. The word count for the revised abstract is 266 words.

9. Only standard abbreviations and acronyms are allowed. A selected list is available online at https://urldefense.com/v3/__http://edmgr.ovid.com/ong/accounts/abbreviations.pdf__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJaTyoh9jA$. 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.

10. The journal does not use the virgule symbol (/) in sentences with words. Please rephrase your text to avoid using "and/or," or similar constructions throughout the text. You may retain this symbol if you are using it to express data or a measurement.
RESPONSE:

We limited using / only for unit of measurement.

11. 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%").
RESPONSE:
We revised the presentation as required.

12. Please review the journal's Table Checklist to make sure that your tables conform to journal style. The Table Checklist is available online
here: https://urldefense.com/v3/__http://edmgr.ovid.com/ong/accounts/table_checklist.pdf__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJa9vi5Lrk$.
RESPONSE:
We have complied with the checklist.

13. 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 https://urldefense.com/v3/__http://links.lww.com/LWW-ES/A48__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJaaUMARpc$. The cost for publishing an article as open access can be found
at https://urldefense.com/v3/__http://edmgr.ovid.com/acd/accounts/ifauth.htm__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJaAejAmjU$.

Please note that 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.
RESPONSE:
We are not interested in open access publication of this manuscript.

14. If you choose to revise your manuscript, please submit your revision through Editorial Manager

at https://urldefense.com/v3/__http://ong.editorialmanager.com__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJa2iC4fSI$. Your manuscript should be uploaded in a word processing format such as Microsoft Word. Your revision's cover letter should include the following:

* A confirmation that you have read the Instructions for Authors (https://urldefense.com/v3/__http://edmgr.ovid.com/ong/accounts/authors.pdf__;!!LIr3w8kk_Xxm!_cFgO041tGEl347YoGeOTGIzVQs-C46xmYheKh1g1B_EuuuJkR8pEjJa4ecJCdI$), and

* A point-by-point response to each of the received comments in this letter.

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.

RESPONSE:

All coauthors contributed to and gave approval of this revision and resubmission.

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 Mar 26, 2020, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Nancy C. Chescheir, MD
Editor-in-Chief

2018 IMPACT FACTOR: 4.965
2018 IMPACT FACTOR RANKING: 7th out of 83 ob/gyn journals

Dear Dr. Sriprasert,

A new revision due date has been entered for the above-referenced submission to Obstetrics & Gynecology: Apr 30, 2020. Please make note of the updated deadline.

Please don't hesitate to contact us with any questions or concerns.

Kind Regards,

Obstetrics & Gynecology