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.*

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

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

Date: Jul 24, 2020
To: "Devin D. Smith" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-20-1731

RE: Manuscript Number ONG-20-1731

Effect of Fasting on Total Bile Acids in Pregnancy

Dear Dr. Smith:

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).

Due to the COVID-19 pandemic, your paper will be maintained in active status for 30 days from the date of this letter. If we have not heard from you by Aug 23, 2020, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1:

Introduction
1. Line 50 references a widely accepted threshold of >10umol/L, according to reference 3 the threshold is 6-10 umol/L for fasting and 10-14 umol/L for non-fasting. Therefore, it is unclear how you chose 10 umol/L as your threshold, please clarify.
2. Line 58 references an internal department study, that has not been published, as the basis of the hypothesis. This reference can be removed as the rest of your introduction is sufficient in explaining the premise of the study.
3. Hypothesis is lower TBA in fasting vs not in asymptomatic women - this is known information, not new. Second hypothesis: fasting levels lower than non- fasting in symptomatic women (again this is known), and that non-fasting levels may be >10 and change the diagnosis - this is the logical outcome of the primary hypothesis, perhaps identify whether fasting or non-fasting should be used to make the diagnosis.

Methods
4. The differences in study methodology between the symptomatic groups is not explained - Standardized meal for asymptomatic patients but not for symptomatic patients; tests within 2 hours of standardized meals for asymptomatic but anytime <8h for symptomatic. The tests used for the asymptomatic and symptomatic cohorts are different. Please explain the reason for these differences
5. Internal lab range is 0-19umol/L due to internal study, but they still pick 10umol/L despite your lab suggesting up to 19umol/L is normal. Different lab assays have different thresholds and these can change clinical practice.
6. Does not mention that charts were reviewed for outcomes and timing of delivery

Results
7. Reports median gestational age at delivery (Line 153, 186). Methods do not mention that this was something you collected. Either remove this result or add the appropriate language in the methods section
8. Would be good to know how many had >14 umol/L (upper limit of normal per expert series) in the non-fasting, and how many had >19umol/L (upper limit of normal per internal lab) in the asymptomatic cohort as this is covered for those who were symptomatic.
9. You document the exclusion of those who developed ICP, but they developed ICP AFTER they had already had their blood drawn and were asymptomatic at the time, so exclusion is not explained? These patients should not be excluded from your analysis as they did not have the diagnosis at the time of enrollment or completion of the study.
10. You explore the changes in diagnosis between fasting and non-fasting states with different cut-offs, the number of changes in diagnosis using different cut-offs across fasting and non-fasting values are clinically relevant but not addressed

in your analysis. It might be worth addressing this as your discussion proposes alternative cut-offs but does not state what they should be.

Discussion
11. Well written, addresses a lot of the limitations of the study appropriately. Explores the fact that outcomes do not change unless TBA are significantly higher. Study does propose that cut offs should be higher but does not specify what - using the data in Table 4 you may be able to discuss some potential pros/cons of higher cutoffs.
12. Unclear whether you recommend fasting vs non-fasting measurements when evaluating for ICP.

Reviewer #2: The objective of this paper is to investigate the difference between fasting and non-fasting total bile acid levels in pregnant women using two cohorts: asymptomatic women and women with symptoms concerning for cholestasis of pregnancy.

1) The end of your precis statement feels confusing as it seems counter to your final conclusion and the more important clinical point. Rather than stating "...often drop below the diagnostic threshold of >10umol/L" (lines 2-3) I would recommend redirecting the emphasis. For example, "Fasting total bile acids were significantly lower than non-fasting total bile acids in both asymptomatic and symptomatic pregnant women and their use may lead to more precise diagnosis of pregnancy complications."

2) More care needs to be taken with respect to your references. In your introduction, I would like the references for these statements to be more inclusive. Lines 40-47 are all statements that I believe should have more than one reference listed. Particularly for the statement in lines 45-47, if you are stating that this is a topic of debate and the diagnostic thresholds used/recommended vary, you should have more than one reference. Additionally, reference 5 is not a strong reference as it is an internet location of what I believe is a reprint from another source that I cannot locate on the Pubmed database. There has been a Cochrane database review on this topic recently in 2019 for example that could be a more appropriate reference (doi: 10.1002/14651858.CD012546.pub2). Similarly, as there is enough literature to support a Cochrane database review, the term "poorly-studied" in line 46 should be edited, perhaps to "debated." Your citation of a similar statement in lines 202-203 in more thorough and appropriate.

Also, with respect to references, you have not properly reflected prior work in this area by only citing Adams et al for your statement in lines 54-55. (Heikkinen published data on this in 1983.)

You use different references for very similar statements in lines 55-57 and lines 86-87 and line 203.
Your statement about SMFM recommendations (lines 212-214) cites two references that do not appear to be SMFM statements or guidelines on the topic.

3) The statement of your hypothesis (lines 67-72) should be condensed and simplified. One possibility, paraphrased, "in both fasting asymptomatic and symptomatic women, fasting levels will be lower than non-fasting levels and this may lead to a different rate of cholestasis diagnoses." In general, it gets very wordy whenever you start to refer to "cross the diagnostic threshold" particularly as your prior introduction and later discussion emphasize that the level for cholestasis diagnosis is not certain. It is more straightforward to reference the diagnosis rate, rather than "cross the diagnostic threshold."

4) While the two cohorts are appropriate described in the Methods section, it is clear that they were differed in time (asymptomatic women were enrolled from March 2018 to July 2019 & symptomatic women from December 2018 to March 2020) these are not sequential time periods per say and the use of that term is confusing (line 10, line 75). Also, the difference in timing of these cohorts was never addressed in the discussion of study limitations. Nor were other differences in the cohorts such as the order of testing results, (fasting levels obtained, then fed in asymptomatic cohort versus fed then fasting levels in most symptomatic women) and the gestational ages at time of testing, discussed in the interpretation of their results.

Also, it is not clearly stated if the symptomatic cohort had the same exclusion criteria as the asymptomatic cohort (multiple gestations and food allergies).

5) The gestational age at second and third trimester testing for the asymptomatic groups should be reported in your results in lines 156-162. I would also appreciate the gestational age of time of evaluation to be included in Table 1 as this information is available and I believe applicable.

6) The symptomatic cohort should have a flow diagram of enrolled patients as well. Figure 1 only addresses one of your cohorts.

Minor points:
-Abstract: In the last line of the results, lines 27-28, please specify which cohort you are describing in this statement (I presume the symptomatic group).

-Lines 43-44, specify iatrogenic, spontaneous or both for the type of preterm birth patients are at risk for.

-Lines 58-60, is this published or unpublished data, please specify.

-Were any details asked about the meal(s) content in the symptomatic cohort (lines 124-129) when they were non-fasting?

-Lines 180-191, Table 4, and Figure 2 investigate the rate of diagnosis with different diagnostic cut-offs. This analysis was not discussed in the methods section. Why were these other possible diagnostic cut-offs used for analysis?

-The information provided in the discussion, lines 216-225, needs to be connected to your study rather than just stated without a discussion of how your adds to this or how this information influences the interpretation of your data.

-Supplemental Table for symptomatic cohort is not referenced in your text.

Reviewer #3: This is a prospective study to report on bile acid levels during pregnancy in women with symptomatic and asymptomatic intrahepatic cholestasis of pregnancy (ICP)!

Main issues:
1- Cut offs are usually decided based on the measurement 97.5th% or some level in asymptomatic patients; did the authors consider utilizing their data to provide a cut off in pregnancy based on the data from asymptomatic patients in the first, second and third trimester!

2- Another important finding that need to be highlighted is the increase of bile salts values with progression of pregnancy! Please add to the conclusion and provide more discussion on that!

3- It seems with the current cut off 82% of symptomatic non fasting patients are positive which is important validation for the current guidelines for treating those patients as higher risk and effect delivery earlier! A conclusion of using the non-fasting or feeding challenged scenario might be another good suggestion based on the results of this study!

Specific issues:
1- Introduction: Can be shorter!

2- Methods:

3- Line 164: this is very important as those asymptomatic patients non-fasting was abnormal and might be a good screen during second trimester (challenge test) that can predict who will get ICP later!

4- Table 1: please add P value comparing asymptomatic compared to symptomatic

5- Figure 1: please include the flow chart for symptomatic patients as well!

6- Figure 2: Please add the control either in the same figure or in another subfigure!

7- Discussion: It might be important to discuss the outcomes of asymptomatic women with higher bile salts as those are not well studied patients!

STATISTICAL EDITOR COMMENTS:

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

Table 1: The n(%) format for the symptomatic should be changed to integer %, not cited to 0.1% precision, since the denominator is only n = 26. Need to include the GA at evaluation for the asymptomatic group.

Tables 2 and 3: Should include the number of women who had fasting/non-fasting measurements at 2nd or 3rd trimesters or include as a footnote that there were no missing data (ie, all rows in Table had n = 27). Could consolidate Tables and indicate the various comparisons in footnote. Should state whether the p = .05 did/did not satisfy the inference test.

Table 4, Fig 2: Should contrast this information with that from the asymptomatic cohort. From Table 2, the upper range of TBA values included some fasting and non-fasting values that were above even the highest TBA threshold of 20 μmol/L.

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 relevant topics. Adherence to these requirements with your revision will avoid delays during the revision process by avoiding re-revisions on your part in order to comply with formatting. For instance, TBA is not an acceptable abbreviation, nor is ICP..

Numbers below refer to line numbers.

9. It's best, I think, to describe this as a report of 2 different studies, not a single study. The methods of the study for the 2 groups was quite different. No standard meal for the symptomatic patients, different gestational ages, different definitions of fasting/non fasting. . It's fine to say this is a report of two different studies, one in asymptomatic women and one in symptomatic women, that are reported together to describe the results of total bile acid testing in the fasting and fed state.

15. In whom fasting ……. Were these women given a standardized meal? Did each woman get fasting and non fasting testing?

21. You can leave the racial make up of the group out of the abstract.

26. These may be statistically significantly different but 11.5 v 13.5 are not really clinically different, do you think?

32. You don't provide the data showing that the asymptomatic women "often" had values exceeding the diagnostic threshold of > 10 in the results. Either delete this from the conclusion or provide it in the results section. Give results, if you choose to do this, for both fasting and non fasting frequency of results > 10.

33. How do you know that the non-fasting results were wrong in the symptomatic patients?

59. Consider "388 women diagnosed with intrahepatic cholestasis of pregnancy of whom approximately…."

Can you show us how many asymptomatic women had fasting levels between 10 and 20
And the results of their fed testing? Its these borderline levels that are really the problem, isn't it?

71. Not sure what you mean by "may cross the diagnostic threshold". Do you mean for symptomatic patients, testing in the fasting state may result in values < 10? Please be specific.

75. Again, this is not a single study, but rather the report of 2 different studies.

76. In whom fasting and non-fasting…

81. So a patient who ate 8 hours and 10 minutes ago was fasting, and one who ate 7 hours 50 minutes ago was non fasting? This is problematic. Do you report the mean times from last food in the symptomatic patients? If not, you need to do so.

86. I don't quite understand your sample size calculation. What end point was this sample size calculated for?

94. Please describe as the first study, not cohort.

117. How was this drop out rate used in the recruitment plan?

133. So there were 3 different labs used? This needs to be in your limitations sections.

151. The AMA style manual, which the Journal uses, asks that "authors provide an explanation of who classified individuals' race, ethnicity, or both, the classifications used, and whether the options were defined by the investigator or the participant. In addition, the reasons that race/ethnicity were assessed in the study also should be described (eg, in the Methods section and/or in table footnotes)."

In addition, the nonspecific "other" as it is sometimes used for comparison in data analysis may also be a "convenience" grouping/label that should be avoided, unless it was a prespecified formal category in a database or research instrument.

Also, White and Black, as racial categories, are now capitalized.

If you retain the description of the racial make up of your study groups, this should be rewritten as "16/27 were Black, 10/27 White and 1/27….".

159. There is considerable overlap in the ranges here across all fed/fasting which is an important observation. Can you tell us how many in the fasting group had values < 10, values > 10, and the same for the fed group? You've partially done this on line 161. Perhaps it would be clearer if written: In the fasting group, xx patients had values <= 10 micromole/L, yy patients had values > 10….. In the non fasting group zz patients had values <= 10 ……"

I would delete lines 164-173 as these women were excluded.

176: see prior notes re: describing race.

179. Similar request as 159 above for reporting values.

181. Instead of "were diagnostic" say were > 10….

I realize this is part of the problem but there is no gold standard for diagnosing cholestasis other than bile acid levels + symptoms. To really make an argument for what values are needed, it seems that you need outcome data and given that the bad outcomes are unusual, it would require a very large study looking at different cut offs to determine a cut off. You argued in your response to the original submission that you think its valuable to avoid iatrogenic early term delivery in over diagnosed patients and I think you need to be very clear that this is your bias in your discussion. Others might reasonably argue that its preferable to avoid a stillbirth and trade that for the early term morbidities, but not mortalities.

194… you did show this but there is a lot of overlap, as noted above.

204. How many asymptomatic women had either fasting or fed values >=20?

220. Same question for TBA levels 40 or more?

If you are looking for a value that should prompt assessment, why not look at this level?

Lines 237-244. I would delete these as you excluded these women from analysis.

252. This doesn't make sense—am I missing something?

"little increased risk with TBA much greater than 10…". What about 100? That's Much greater than 10 and there seems to be significantly increased risks for these patients.

Please provide a table for the asymptomatic patients, similar to what you've provided w/ the data for the symptomatic patients, in supplemental digital content.

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. Obstetrics & Gynecology uses an "electronic Copyright Transfer Agreement" (eCTA). 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. For studies that report on the topic of race, authors must provide an explanation in the manuscript of who classified individuals' race, ethnicity, or both, the classifications used, and whether the options were defined by the investigator or the participant. In addition, the reasons that race/ethnicity were assessed in the study also should be described (eg, in the Methods section and/or in table footnotes).

Use "Black" and "White" (capitalized) when used to refer to racial categories.

The category of "Other" is a grouping/label that should be avoided, unless it was a prespecified formal category in a database or research instrument. If you use "Other" in your study, please add detail to the manuscript to describe which patients were included in that category.

5. Please submit a completed CONSORT checklist.

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), observational studies using ICD-10 data (ie, RECORD), 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, RECORD, CHEERS, SQUIRE 2.0, or CHERRIES guidelines, as appropriate.

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

7. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Original Research reports should not exceed 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.

8. 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).

9. 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 limit for Original Research articles is 300 words. Please provide a word count.

10. 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.

11. 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.

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

13. 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%").

14. 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.

15. The American College of Obstetricians and Gynecologists' (ACOG) documents are frequently updated. These documents may be withdrawn and replaced with newer, revised versions. If you cite ACOG documents in your manuscript, be sure the reference you are citing is still current and available. If the reference you are citing has been updated (ie, replaced by a newer version), please ensure that the new version supports whatever statement you are making in your manuscript and then update your reference list accordingly (exceptions could include manuscripts that address items of historical interest). If the reference you are citing has been withdrawn with no clear replacement, please contact the editorial office for assistance (obgyn@greenjournal.org). In most cases, if an ACOG document has been withdrawn, it should not be referenced in your manuscript (exceptions could include manuscripts that address items of historical interest). All ACOG documents (eg, Committee Opinions and Practice Bulletins) may be found at the Clinical Guidance page at https://www.acog.org/clinical (click on "Clinical Guidance" at the top).

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

17. Each supplemental file in your manuscript should be named an "Appendix," numbered, and ordered in the way they are first cited in the text. Do not order and number supplemental tables, figures, and text separately. References cited in appendixes should be added to a separate References list in the appendixes file.

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

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.

***

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 30 days from the date of this letter. If we have not heard from

you by Aug 23, 2020, we will assume you wish to withdraw the manuscript from further consideration.***.

Sincerely,

Nancy C. Chescheir, MD
Editor-in-Chief

2019 IMPACT FACTOR: 5.524
2019 IMPACT FACTOR RANKING: 6th out of 82 ob/gyn journals

THE OHIO STATE UNIVERSITY WEXNER MEDICAL CENTER

Division of
Maternal Fetal Medicine

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER

September 9, 2020

To The Editor,

Obstetrics and Gynecology

Enclosed is our manuscript entitled, "Effect of Fasting on Total Bile Acids in Pregnancy," submitted for publication in *Obstetrics and Gynecology* following an invitation to revise our previous manuscript. In this manuscript, we describe two prospective cohort studies evaluating differences between fasting and non-fasting total bile acids in pregnant women. With our data, we demonstrate that fasting total bile acids are significantly lower than non-fasting total bile acids in asymptomatic and symptomatic pregnant women. Additionally, we found that non-fasting total bile acids in asymptomatic women exceeded 10 µmol/L, often greatly, and that nearly 25% of symptomatic women with non-fasting total bile acids >10 µmol/L had fasting total bile acids ≤10 µmol/L.

We greatly appreciate the feedback from our reviewers and, following their advice, have revised our manuscript substantially. We have copied below the comments received on the prior manuscript, all of which have been addressed.

We have complied with the journal's submission guidelines and within our document we have included a title page, manuscript body, references, tables, and figure captions. I affirm 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. I also affirm that this manuscript is not under consideration elsewhere and will not be submitted to another journal until a final decision has been made by the editors of *Obstetrics and Gynecology.*

All the authors provided substantial scientific input to the drafting or revision of this manuscript with regard to scientific content and form and approved the final manuscript as submitted. There are no financial interests, commercial affiliations, or other conflicts of interest.

On behalf of my co-authors, I thank you for considering this manuscript for publication.

Respectfully,
Devin D. Smith, MD
Fellow in the Division of Maternal-Fetal Medicine
Department of Obstetrics and Gynecology
The Ohio State University, College of Medicine
[redacted]

THE OHIO STATE UNIVERSITY WEXNER MEDICAL CENTER
Division of
Maternal Fetal Medicine
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER

RE: Manuscript Number ONG-20-1731

Effect of Fasting on Total Bile Acids in Pregnancy

REVIEWER COMMENTS:

Reviewer #1:

Introduction

1. Line 50 references a widely accepted threshold of >10umol/L, according to reference 3 the threshold is 6-10 umol/L for fasting and 10-14 umol/L for non-fasting. Therefore, it is unclear how you chose 10 umol/L as your threshold, please clarify.

Thank you for noting this. In the United States, 10 umol/L is often used as the clinical diagnostic threshold. This holds true in the majority of studies evaluating bile acids in pregnancy. We have included an additional citation to a recent Cochrane Review (July 2019) which emphasizes this point.

2. Line 58 references an internal department study, that has not been published, as the basis of the hypothesis. This reference can be removed as the rest of your introduction is sufficient in explaining the premise of the study.

Thank you for your comment. Following the recommendation of another reviewer, we have specified that this is unpublished data in the body of the manuscript.

3. Hypothesis is lower TBA in fasting vs not in asymptomatic women - this is known information, not new. Second hypothesis: fasting levels lower than non- fasting in symptomatic women (again this is known), and that non-fasting levels may be >10 and change the diagnosis - this is the logical outcome of the primary hypothesis, perhaps identify whether fasting or non-fasting should be used to make the diagnosis.

Thank you for your comment. While it is known that fasting TBA are lower than postprandial TBA, this has not been widely studied or confirmed in pregnant women. With our data we hope to add to a very limited aspect of the literature regarding ICP. While we would like to use our data to identify whether fasting or non-fasting should be used to make the diagnosis, our data is limited and previous reviewers have recommended we be cautious with our conclusions.

Methods

4. The differences in study methodology between the symptomatic groups is not explained - Standardized meal for asymptomatic patients but not for symptomatic patients; tests within 2 hours of standardized meals for asymptomatic but anytime <8h for symptomatic. The tests used for the asymptomatic and symptomatic cohorts are different. Please explain the reason for these differences.

Thank you for your comment. In the manuscript we mentioned that "In the symptomatic cohort a pragmatic design was chosen to mimic clinical practice and the non-fasting state was not controlled." Data from two separate cohorts is being presented together under the recommendation of previous reviewers. We chose a different test in the symptomatic cohort (random TBA rather than controlled postprandial) in the hopes that it would most closely resemble what was being done clinically.

5. Internal lab range is 0-19umol/L due to internal study, but they still pick 10umol/L despite your lab suggesting up to 19umol/L is normal. Different lab assays have different thresholds and these can change clinical practice.

Thank you for your comment. Our institutional lab's reference range is derived from an internal study of a healthy non-pregnant mixed-sex population. Our department does not believe a non-pregnant population that includes men to be generalizable to the obstetric population. A threshold of 10 was adopted in accordance with obstetrics literature.

6. Does not mention that charts were reviewed for outcomes and timing of delivery.

Thank you for noting this. We have added a line to the methods to make this clear.

Results

7. Reports median gestational age at delivery (Line 153, 186). Methods do not mention that this was something you collected. Either remove this result or add the appropriate language in the methods section.

Thank you for noting this. We have added a line to the methods to make this clear.

8. Would be good to know how many had >14 umol/L (upper limit of normal per expert series) in the non-fasting, and how many had >19umol/L (upper limit of normal per internal lab) in the asymptomatic cohort as this is covered for those who were symptomatic.

Thank you for your comment. This was also recommended by other reviewers and, as such, we have provided this information in Table 2 as well as in supplementary Table 3.

9. You document the exclusion of those who developed ICP, but they developed ICP AFTER they had already had their blood drawn and were asymptomatic at the time, so exclusion is not explained? These patients should not be excluded from your analysis as they did not have the diagnosis at the time of enrollment or completion of the study.

Thank you for your comment. Though we initially agreed, we were asked by previous reviewers to exclude those patients from the analysis. We understand and appreciate both recommendations. The significance of the results did not change after their exclusion.

10. You explore the changes in diagnosis between fasting and non-fasting states with different cut-offs, the number of changes in diagnosis using different cut-offs across fasting and non-fasting values are clinically relevant but not addressed in your analysis. It might be worth addressing this as your discussion proposes alternative cut-offs but does not state what they should be.

Thank you for your comment. The authors agree that number of changed diagnoses at the different cutoffs is clinically relevant and have included those numbers in the results. However, given our small sample size the absolute numbers of changed diagnoses are small (6 patients at >10, 5 at >15, and 1 at >20) and thus difficult to compare. Additionally, previous reviewers recommended that we be cautious in our conclusions/recommendations for new cutoffs.

Discussion

11. Well written, addresses a lot of the limitations of the study appropriately. Explores the fact that outcomes do not change unless TBA are significantly higher. Study does propose that cut offs should be higher but does not specify what - using the data in Table 4 you may be able to discuss some potential pros/cons of higher cutoffs.

Thank you for your comment. We are very glad that you found our discussion well-written. We initially were prescriptive in our discussion/recommendation for higher cutoffs. However, we were advised not to draw strong conclusion from our data as the sample was small. While we believe that a threshold of 20 would be reasonable and would decrease the number of potentially changed diagnoses between the fasting and non-fasting state, we agree that our data is too limited to make such a recommendation.

12. Unclear whether you recommend fasting vs non-fasting measurements when evaluating for ICP.

Thank you for your comment. We were purposefully vague in our recommendations for the reasons listed above. We mention that, “our findings underscore the potential importance of collecting TBA in the fasting state when evaluating symptoms concerning for ICP….[and] suggest that fasting evaluation of TBA or a higher threshold for diagnosis of ICP should be considered.” In the clinical setting, it is often difficult to collect fasting bile acids and as such, a higher threshold may be more appropriate.

Reviewer #2: The objective of this paper is to investigate the difference between fasting and non-fasting total bile acid levels in pregnant women using two cohorts: asymptomatic women and women with symptoms concerning for cholestasis of pregnancy.

1) The end of your precis statement feels confusing as it seems counter to your final conclusion and the more important clinical point. Rather than stating "...often drop below the diagnostic threshold of >10umol/L" (lines 2-3) I would recommend redirecting the emphasis. For example, "Fasting total bile acids were significantly lower than non-fasting total bile acids in both asymptomatic and symptomatic pregnant women and their use may lead to more precise diagnosis of pregnancy complications."

Thank you for your comment. The precis has been updated as such.

2) More care needs to be taken with respect to your references. In your introduction, I would like the references for these statements to be more inclusive. Lines 40-47 are all statements that I believe should have more than one reference listed. Particularly for the statement in lines 45-47, if you are stating that this is a topic of debate and the diagnostic thresholds used/recommended vary, you should have more than one reference. Additionally, reference 5 is not a strong reference as it is an internet location of what I believe is a reprint from another source that I cannot locate on the Pubmed database. There has been a Cochrane database review on this topic recently in 2019 for example that could be a more appropriate reference (doi: 10.1002/14651858.CD012546.pub2). Similarly, as there is enough literature to support a Cochrane database review, the term "poorly-studied" in line 46 should be edited, perhaps to "debated." Your citation of a similar statement in lines
202-203 in more thorough and appropriate.

Thank you for your comment. We agree and have included additional and more specific references for the statements made in the first paragraph of the introduction. We have also edited the term “poorly-studied” as suggested.

Also, with respect to references, you have not properly reflected prior work in this area by only citing Adams et al for your statement in lines 54-55. (Heikkinen published data on this in 1983.)

Thank you for your comment. We have updated the citation to include the work by Heikkinen.

You use different references for very similar statements in lines 55-57 and lines 86-87 and line 203.

Thank you for your comment. We agree that the citations for those statements (which are similar) should be consistent and more robust. We have updated said citations to match each other and also include additional references.

Your statement about SMFM recommendations (lines 212-214) cites two references that do not appear to be SMFM statements or guidelines on the topic.

Thank you for your comment. The authors struggled with how to present this information. A query of the SMFM publications website provides only one article about cholestasis, a 2011 article from Contemporary Ob/Gyn by author Craigo. We agree that this finding does not equate to an SMFM statement or guideline and have updated this in the text. We do, however, find it important to highlight the endorsement SMFM gives this article by including it on their publications page. We have updated the manuscript as such. “Though its role and utility remains controversial, antenatal testing is seemingly endorsed by the Society for Maternal-Fetal Medicine (SMFM) for pregnancies complicated by ICP. However, authors of the sole article regarding cholestasis listed on the SMFM publications webpage recognize that the optimal type, duration, and frequency of antenatal testing is unknown.[10,24]”

3) The statement of your hypothesis (lines 67-72) should be condensed and simplified. One possibility, paraphrased, "in both fasting asymptomatic and symptomatic women, fasting levels will be lower than non-fasting levels and this may lead to a different rate of cholestasis diagnoses." In general, it gets very wordy whenever you start to refer to "cross the diagnostic threshold" particularly as your prior introduction and later discussion emphasize that the level for cholestasis diagnosis is not certain. It is more straightforward to reference the diagnosis rate, rather than "cross the diagnostic threshold."

Thank you for your comment. We agree and have updated this in the manuscript.

4) While the two cohorts are appropriate described in the Methods section, it is clear that they were differed in time (asymptomatic women were enrolled from March 2018 to July 2019 & symptomatic women from December 2018 to March 2020) these are not sequential time periods per say and the use of that term is confusing (line 10, line 75). Also, the difference in timing of these cohorts was never addressed in the discussion of study limitations. Nor were other differences in the cohorts such as the order of testing results, (fasting levels obtained, then fed in asymptomatic cohort versus fed then fasting levels in most symptomatic women) and the gestational ages at time of testing, discussed in the interpretation of their results.

Thank you for your comments. The term sequential has been removed as recommended. We have also added/addressed the recommendation to include the timing of the two cohorts in our limitations. “An additional limitation relates to the timing of the two cohorts. The decision to include a second cohort of symptomatic women was made after the initial study of asymptomatic women had commenced. Ideally, these two cohorts would have been planned as part of one sequential study. We chose to present both cohorts together given the contextual and clinical relationship to one another.” Because the cohorts were two different studies, the methodology for each was different. We did not discuss the different methodologies as part of the limitations because we did not make comparisons between the two.

Also, it is not clearly stated if the symptomatic cohort had the same exclusion criteria as the asymptomatic cohort (multiple gestations and food allergies).

Thank you for your comment. We have clarified that multiple gestation was an exclusion criteria for both. Given that we did not control the fed state in the symptomatic group (they were not given a standardized meal), lactose intolerance or food allergy were not exclusion criteria.

5) The gestational age at second and third trimester testing for the asymptomatic groups should be reported in your results in lines 156-162. I would also appreciate the gestational age of time of evaluation to be included in Table 1 as this information is available and I believe applicable.

Thank you for your comment. The gestational age at evaluation has been added to the aforementioned results section as well as Table 1.

6) The symptomatic cohort should have a flow diagram of enrolled patients as well. Figure 1 only addresses one of your cohorts.

Thank you for your comment. An additional flow diagram has been included.

Minor points:
-Abstract: In the last line of the results, lines 27-28, please specify which cohort you are describing in this statement (I presume the symptomatic group).

Thank you for your comment, this has been updated.

-Lines 43-44, specify iatrogenic, spontaneous or both for the type of preterm birth patients are at risk for.

Thank you for your comment, this has been updated to clarify both spontaneous and iatrogenic preterm birth.

-Lines 58-60, is this published or unpublished data, please specify.

Thank you for your comment, we have specified that this is unpublished data.

-Were any details asked about the meal(s) content in the symptomatic cohort (lines 124-129) when they were non-fasting?

Thank you for your comment. Unfortunately, we did not elicit details regarding the content of last meal in the symptomatic cohort. We have added this to our limitations.

-Lines 180-191, Table 4, and Figure 2 investigate the rate of diagnosis with different diagnostic cut-offs. This analysis was not discussed in the methods section. Why were these other possible diagnostic cut-offs used for analysis?

Thank you for your comment. We included this analysis (referred to as descriptive statistics in the Methods section) with the intent of highlighting the rate of diagnosis in our cohort based on cut-offs used in the literature. We feel that highlighting this adds to our manuscript and supports our conclusion that “our findings suggest that fasting evaluation of TBA or a higher threshold for diagnosis of ICP should be considered.”

-The information provided in the discussion, lines 216-225, needs to be connected to your study rather than just stated without a discussion of how your adds to this or how this information influences the interpretation of your data.

Thank you for your comment, we have updated this section of the manuscript with a discussion of how our study related to the Ovadia meta-analysis.

-Supplemental Table for symptomatic cohort is not referenced in your text.

Thank you for your comment, we have referenced the Supplement Table in the manuscript.

Reviewer #3: This is a prospective study to report on bile acid levels during pregnancy in women with symptomatic and asymptomatic intrahepatic cholestasis of pregnancy (ICP)!

Main issues:
1- Cut offs are usually decided based on the measurement 97.5th% or some level in asymptomatic patients; did the authors consider utilizing their data to provide a cut off in pregnancy based on the data from asymptomatic patients in the first, second and third trimester!

Thank you for your comment. Unfortunately, our sample size is too small to provide reference ranges or cut-offs for the general population.

2- Another important finding that need to be highlighted is the increase of bile salts values with progression of pregnancy! Please add to the conclusion and provide more discussion on that!

Thank you for your comment. We have added this to our discussion.

3- It seems with the current cut off 82% of symptomatic non fasting patients are positive which is important validation for the current guidelines for treating those patients as higher risk and effect delivery earlier! A conclusion of using the non-fasting or feeding challenged scenario might be another good suggestion based on the results of this study!

Thank you for your comment. While 81% of the symptomatic patients would have received a diagnosis of ICP using non-fasting TBA, many of those patients (23%) would not have been diagnosed had fasting TBA been used for diagnosis. Given recent and robust data indicating that women with relatively low TBA are not actually high risk, we believe our data argues that fasting evaluation of TBA or a higher threshold for diagnosis of ICP should be considered.

Specific issues:
1- Introduction: Can be shorter!

Thank you for your comment. We have shortened our introduction.

2- Methods: (no comment)

3- Line 164: this is very important as those asymptomatic patients non-fasting was abnormal and might be a good screen during second trimester (challenge test) that can predict who will get ICP later!

Thank you for your comment. We agree that this is an interesting finding. However, the non-fasting values were "abnormal" across the entire cohort with median 2nd trimester non-fasting 13.62 (2.03-40.26) and median 3rd trimester non-fasting 17.35 (1.77-62.93).

4- Table 1: please add P value comparing asymptomatic compared to symptomatic

Thank you for your comment. We chose not to include p-values in our demographics table in accordance with recent trends in medical statistics/the literature.

5- Figure 1: please include the flow chart for symptomatic patients as well!

Thank you for your comment. We have included a flow chart for symptomatic patients.

6- Figure 2: Please add the control either in the same figure or in another subfigure!

Thank you for this suggestion, however we are a bit unclear of what the reviewer is asking regarding controls to be displayed in the figure. The figure demonstrates the proportion of patients who were diagnosed with ICP at different diagnostic thresholds. The relationship with the proportion of patients not diagnosed with ICP is the inverse and thus implied (ie if 81% of patients were diagnosed then 19% were not). We are more than happy to make further edits if necessary.

7- Discussion: It might be important to discuss the outcomes of asymptomatic women with higher bile salts as those are not well studied patients!

Thank you for your comment. While that is very interesting, discussion of outcomes is outside the scope of this study.

STATISTICAL EDITOR COMMENTS:

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

Table 1: The n(%) format for the symptomatic should be changed to integer %, not cited to 0.1% precision, since the denominator is only n = 26. Need to include the GA at evaluation for the asymptomatic group.

Thank you for your comment. This has been updated.

Tables 2 and 3: Should include the number of women who had fasting/non-fasting measurements at 2nd or 3rd trimesters or include as a footnote that there were no missing data (ie, all rows in Table had

n = 27). Could consolidate Tables and indicate the various comparisons in footnote. Should state whether the p = .05 did/did not satisfy the inference test.

Thank you for your comment. We have added the number of samples (rather than patients, given that each patient ideally would have had 4 samples) for each category. We have also added an explanation of the missing samples in the footnotes of each table. And lastly, we have states that p = 0.05 did satisfy the test for inference.

Table 4, Fig 2: Should contrast this information with that from the asymptomatic cohort. From Table 2, the upper range of TBA values included some fasting and non-fasting values that were above even the highest TBA threshold of 20 µmol/L.

Thank you for your comment. We, too, find this information very interesting. However, given the different patient populations and study methodologies between the two cohorts we were reticent to make any comparisons between the two.

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 relevant topics. Adherence to these requirements with your revision will avoid delays during the revision process by avoiding re-revisions on your part in order to comply with formatting. For instance, TBA is not an acceptable abbreviation, nor is ICP..

Thank you for your comment. We will be sure to adhere to Green Journal formatting requirements with our revision.

Numbers below refer to line numbers.

9. It's best, I think, to describe this as a report of 2 different studies, not a single study. The methods of the study for the 2 groups was quite different. No standard meal for the symptomatic patients, different gestational ages, different definitions of fasting/non fasting. . It's fine to say this is a report of two different studies, one in asymptomatic women and one in symptomatic women, that are reported together to describe the results of total bile acid testing in the fasting and fed state.

Thank you for your comment. We struggled with how to present these two different but related cohorts and appreciate your suggestion. We have updated this and other related portions of the manuscript to reflect the change. Specifically, we have changed the opening to the Methods section of the abstract to read "This is a report of two prospective cohort studies describing total bile acid levels in the fasting and non-fasting state in pregnancy. The first cohort included…"

15. In whom fasting ……. Were these women given a standardized meal? Did each woman get fasting and non fasting testing?

Thank you for your comment. The specific methodology regarding controlled non-fasting state (standardized meal) vs. uncontrolled non-fasting state (no standardized meal) is clarified in the body of the manuscript. We chose to leave this out of the abstract to keep it simple and within the word count limit.

21. You can leave the racial make up of the group out of the abstract.

Thank you for your comment. We have removed the racial makeup from the abstract.

26. These may be statistically significantly different but 11.5 v 13.5 are not really clinically different, do you think?

Thank you for your comment. While the difference between the two is small, we do believe the difference is clinically significant given the proximity of the values to the diagnostic threshold.

32. You don't provide the data showing that the asymptomatic women "often" had values exceeding the diagnostic threshold of > 10 in the results. Either delete this from the conclusion or provide it in the results section. Give results, if you choose to do this, for both fasting and non fasting frequency of results > 10.

Thank you for your comment. This information is provided in the body of the manuscript but, following your recommendation, we have added it to the results section of the abstract as well. Specifically, we added that "TBA exceeded 10 µmol/L in 20% of the fasting samples and in 56% of the non-fasting samples in the 3rd trimester."

33. How do you know that the non-fasting results were wrong in the symptomatic patients?

Thank you for your comment. We do not know that non-fasting results are "wrong" with regard to diagnosis. We acknowledge that the standard of practice at our institution is, in fact, to use the non-fasting results to guide management. Our main goal with our data and conclusions is to highlight that fasting levels are reliably and significantly lower than non-fasting levels and that diagnostic thresholds vary and are sometimes based upon fasting reference values.

59. Consider "388 women diagnosed with intrahepatic cholestasis of pregnancy of whom approximately…."

Thank you for your comment. This has been updated in the manuscript.

Can you show us how many asymptomatic women had fasting levels between 10 and 20And the results of their fed testing? Its these borderline levels that are really the problem, isn't it?

Thank you for your comment. Yes, the borderline levels are what we are attempting to highlight as potential areas for changes in management. We have added a column to Table 2 showing the frequency of TBA >10 µmol/L and TBA 11-20 µmol/L.

71. Not sure what you mean by "may cross the diagnostic threshold". Do you mean for symptomatic patients, testing in the fasting state may result in values < 10? Please be specific.

Thank you for your comment. Following a similar recommendation from another reviewer, we have revised that sentence to read "We hypothesized that fasting TBA, in both asymptomatic and symptomatic women, will be lower than non-fasting levels and this may lead to a different rate of cholestasis diagnoses."

75. Again, this is not a single study, but rather the report of 2 different studies.

Thank you for your comment, this has been revised accordingly.

76. In whom fasting and non-fasting…

Thank you for your comment, this has been revised accordingly.

81. So a patient who ate 8 hours and 10 minutes ago was fasting, and one who ate 7 hours 50 minutes ago was non fasting? This is problematic. Do you report the mean times from last food in the symptomatic patients? If not, you need to do so.

Thank you for your comment. We greatly appreciate you finding this error in our manuscript. For the symptomatic cohort, fasting was defined similarly to the asymptomatic cohort (nothing per mouth for at least 8 hours). However, non-fasting was defined as any oral intake within the last 4 hours, not 8. This was done precisely to avoid the scenario which you describe. All patients in that cohort had non-fasting samples drawn within 4 hours of oral intake and repeat non-fasting samples were not necessary in any patients.

86. I don't quite understand your sample size calculation. What end point was this sample size calculated for?

Thank you for your comment. The end point was total bile acid levels with estimations for fasting and non-fasting taken from exisinting literature.

94. Please describe as the first study, not cohort.

Thank you for your comment, this has been revised accordingly.

117. How was this drop out rate used in the recruitment plan?

Thank you for your comment. Because of the predicted high drop-out rate, we aimed to enroll at least twice the sample size needed for the study. We used twice the calculated sample size based on dropout rates from previous studies performed with sample population. We have added this to the Methods.

133. So there were 3 different labs used? This needs to be in your limitations sections.

Thank you for your comment. Only one lab was used for each study, though it was not the same lab. For the first study, we ran samples in our department lab. For the second study, all samples were run by the same Quest Diagnostics lab which our hospital utilized for bile acid evaluation. We have addressed this limitation in the manuscript.

151. The AMA style manual, which the Journal uses, asks that "authors provide an explanation of who classified individuals' race, ethnicity, or both, the classifications used, and whether the options were defined by the investigator or the participant. In addition, the reasons that race/ethnicity were assessed in the study also should be described (eg, in the Methods section and/or in table footnotes)."

In addition, the nonspecific "other" as it is sometimes used for comparison in data analysis may also be a "convenience" grouping/label that should be avoided, unless it was a prespecified formal category in a database or research instrument.

Also, White and Black, as racial categories, are now capitalized.

If you retain the description of the racial make up of your study groups, this should be rewritten as "16/27 were Black, 10/27 White and 1/27….".

Thank you for your comment. We have revised the manuscript accordingly. Specifically we have added to the Methods section "Charts were reviewed for demographic and outcome information. Race options were defined by prespecified formal categories in the institutional electronic medical record system and chosen by participants independent of the study." We have also added the footnote to table 1 "race was chosen as a demographic variable given the known ethnic variation in rates of cholestasis diagnoses."

159. There is considerable overlap in the ranges here across all fed/fasting which is an important observation. Can you tell us how many in the fasting group had values < 10, values > 10, and the same for the fed group? You've partially done this on line 161. Perhaps it would be clearer if written: In the fasting group, xx patients had values <= 10 micromole/L, yy patients had values > 10….. In the non fasting group zz patients had values <= 10 ……"

Thank you for your comment. We have added the requested information to Table 2 but would prefer to break the numbers down by trimester first and then fasting/non-fasting as we believe the 3rd trimester numbers are most relevant (given that cholestasis is most often diagnosed in the 3rd trimester).

I would delete lines 164-173 as these women were excluded.

Thank you for your comment. Previous reviewers have made various recommendations regarding whether we should include or exclude these patients and whether we should provide details about their TBA/outcomes or not. Ultimately, following the recommendation of prior reviewers, we chose to exclude them but include their information. We believe that readers will be curious about the information.

176: see prior notes re: describing race.

Thank you for your comment, this has been revised accordingly.

179. Similar request as 159 above for reporting values.

Thank you for your comment. This information is available in Table 4.

181. Instead of “were diagnostic” say were > 10….

Thank you for your comment, this has been revised accordingly.

I realize this is part of the problem but there is no gold standard for diagnosing cholestasis other than bile acid levels + symptoms. To really make an argument for what values are needed, it seems that you need outcome data and given that the bad outcomes are unusual, it would require a very large study looking at different cut offs to determine a cut off. You argued in your response to the original submission that you think its valuable to avoid iatrogenic early term delivery in over diagnosed patients and I think you need to be very clear that this is your bias in your discussion. Others might reasonably argue that its preferable to avoid a stillbirth and trade that for the early term morbidities, but not mortalities.

Thank you for your comment. We agree that large scale outcome data is needed to support any conclusions regarding the diagnostic threshold for cholestasis. We also acknowledge that the largest scale data to date demonstrates a risk of stillbirth similar to background risk for bile acids less than 40. However, to soften our biases regarding antenatal testing and early delivery we have removed mention of both from the closing statement of our discussion.

194… you did show this but there is a lot of overlap, as noted above.

Thank you for your comment.

204. How many asymptomatic women had either fasting or fed values >=20?

Thank you for your comment. Following your recommendation, we have included an additional supplement table with all TBA values by subject tin the asymptomatic group.

220. Same question for TBA levels 40 or more? If you are looking for a value that should prompt assessment, why not look at this level?

Thank you for your comment. Following your recommendation, we have included an additional supplement table with all TBA values by subject tin the asymptomatic group. With regard to using 40 as a TBA value that prompts assessment, we agree in the utility of evaluating 40 as a diagnostic threshold. However, our study question was aimed at evaluating the threshold of 10 and the number of subjects with TBA >40 was very small.

Lines 237-244. I would delete these as you excluded these women from analysis.

Thank you for your comment. Previous reviewers have made various recommendations regarding whether we should include or exclude these patients and whether we should provide details about their TBA/outcomes or not. Ultimately, following the recommendation of prior reviewers, we chose to exclude them but include their information. We believe that readers will be curious about the information and, as such, prefer to address it as a limitation.

252. This doesn't make sense—am I missing something?

"little increased risk with TBA much greater than 10…". What about 100? That's Much greater than 10 and there seems to be significantly increased risks for these patients.

Thank you for your comment. We agree that sentence is poorly worded and have revised it to read "In the wake of recent data demonstrating little increased fetal risk with TBA levels below 40 µmol/L and keeping in mind the risks of antenatal testing and iatrogenic preterm and early term delivery, our findings suggest that fasting evaluation of TBA or a higher threshold for diagnosis of ICP should be considered."

Please provide a table for the asymptomatic patients, similar to what you've provided w/ the data for the symptomatic patients, in supplemental digital content.

Thank you for your comment, we have included an additional supplemental table with this information.