NOTICE: This document contains correspondence generated during peer review and subsequent revisions but before transmittal to production for composition and copyediting:

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

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

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

Questions about these materials may be directed to the Obstetrics & Gynecology editorial office: obgyn@greenjournal.org.
RE: Manuscript Number ONG-19-816

The fetus, not the mother, may provide inflammatory signals required for triggering parturition

Dear Dr. Menon:

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 Jun 21, 2019, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1: This is a cross sectional study of amniotic fluid (AF), cord blood (CB) and maternal plasma (MP) with singleton pregnancies to examine inflammatory mediators to theorize the contribution to parturition. Women had 1 of 4 conditions: term labor, term not in labor, spontaneous PTB, and PPROM. HMGB1, IL-6, and CRP were measured in all three compartments. Amniotic fluid HMGB1, Uric Acid, IL-6 differed, but not CRP. All cord blood measures differed among the groups. In MP, only IL-6 differed. The authors conclude that accumulation of HMBG1 and an increase in general inflammation in AF, on the fetal side, may be the signals of parturition. This observation may lead to discovery of targets for interventions. Ways in which this manuscript could be improved include:

Line 111: Why did you choose 36w0d rather than 36w6d?

Lines 374-375: Did cost limit your study size or enrollment?

Line 402: Does not human data exist? Other mammals?

Reviewer #2: There have been numerous publications looking at the role of inflammatory mediators in parturition, both term and preterm. There are a handful of studies attempting to elucidate the source of the inflammatory "trigger" which leads to labor. Mendolsohn and colleagues looked at one mechanism by which the fetus may contribute to labor (J Steroid Biochem Mol Biol 2017 06;170:19-27), but their target differed from the target in the present study. Menon and colleagues have conducted a cross-section study of three inflammatory marker concentrations in three feto-maternal compartments in women from a previous study who had either pPROM, preterm labor, term labor, or term not in labor (cesarean deliveries). The identification of HMGB1 as higher in amniotic fluid in laboring patients (term labor and preterm labor) with no change in maternal plasma HMGB1 levels suggests that the fetus is the source of elevated HMGB1.

Thus, this paper adds to a growing body of literature which works to identify the physiology surrounding normal labor. This is an necessary topic which may be highly sited.

The results section does require some review, as it is very difficult to follow. There may be a better way to present the analyses described, as well as to describe what is and is not statistically significant. One method may be to alter the description of each figure to explain. Similarly, the methods section regarding statistics is equally difficult to follow.
Reviewer #3: In this manuscript, the authors assess the level of HMGB1, uric acid, IL-6, and CRP in the maternal blood, cord blood, and amniotic fluid. They found differing levels of HMGB1, uric acid, and IL-6 as a result of this study.

I have several comments:
1) The methods section is much too long and needs to be edited down (8 pages long in its current format). For example, it is not necessary to list the procedure for the HMGB1 ELISA kit—rather just state HMGB1 was analyzed according to the manufacturer's protocol.

2) The statistical analysis of data is over 3 pages long, which again is much too long and needs to be edited down.

3) The manuscript discusses how an accumulation of fetal inflammatory markers is associated with preterm birth. This has been published previously as far back as 1998 and I'm not sure if this descriptive study adds to this prior work:
The fetal inflammatory response syndrome

4) I am not sure if this is an appropriate journal for this manuscript.

Reviewer #4:

Title: The title is appropriate for the review, and appropriate in length.

Summary:
This is a cross-sectional study assessing whether inflammatory markers from amniotic fluid, cord blood, and maternal plasma were associated with parturition.

Abstract:
Objective section: while the objective is appropriate it could be made clearer if authors were more specific. If the authors sought to determine whether mature fetal organs released inflammatory biomarkers that triggered parturition by examining biomarkers released in three feto-maternal compartments in women at term and preterm, it is not clear from the objective as written. This is not mentioned until the end of the introduction section, making it difficult for a reader to glean from the abstract.

Methods section (lines 34-41): The abstract does not state that a parent study was used to conduct the cross-sectional analysis. The time period during which samples were taken, and the number required to be powered to see a significant difference between the groups was not stated. Why was the design unbalanced if this was taken from a larger cohort of patients? The analytes being compared are also non-specific and can differ per person at baseline.

Results section (lines 42-49): this section is unclear and confusing to the reader. It states that all amniotic fluid analytes and cord blood analytes (in line 43-44) differed across groups. The author then writes "in contrast, only maternal plasma IL-6 differed across groups". It is not clear whether the author is referring to the different labor groups or the different compartments being compared throughout this section. It may be easier to interpret the findings if the authors explained the findings as they relate to women who were term not in labor, term in labor, preterm in labor, and women with PPROM.

Line 47: The statement "IL-6 and uric acid were also elevated in the term labor compared to other conditions", which compartment is this referring to?

Conclusion (lines 50-53): The authors state that HMGB1 and an increase in inflammatory markers on the fetal side instead of the maternal side. However, HMGB1 was not found to be increased in preterm labor per the results section. If the hypothesis is that mature fetal organs release biochemical mediators into the uterine environment, that is not clear from the results or the conclusion.

In addition, it is difficult to associate an increase in inflammatory markers in PPROM due to some underlying infectious process that is not identified. Without comparing analytes before and during labor in each person per condition, it is difficult to conclude that the differences in analytes noted in the fetal compartments are truly representative of parturition.

Introduction (lines 58-96):
This section of the manuscript is well written and provides good reasoning for the study question. However, it is not clear why the particular biomarkers of HMGB-1, uric acid, IL-6 and CRP were chosen to be examined for this study.

Materials and Methods:
Lines (98-100): while the authors obtained their cohort from a parent study recruited from 2003-2011 to study racial disparities in biomarker profiles that contribute to preterm birth, the women selected for this study were predominantly white and only selected between the years 2008-2010, limiting the generalizability of the study. It is also not clear why these specific years were selected. How many patients were in the parent study and how was the subset of patients from the parent study selected? Was a flow diagram designed to show how subjects were included and excluded in the current study from the parent study?

Line 111: Is there a reason for changing the definition of preterm delivery to <36 weeks 0 days from <37 weeks?

Lines 127-138: Was the same person collecting these samples? Could obtaining amniotic fluid after uterine incision or anesthesia and antibiotics received during a cesarean potentially change the biomarkers that could be present in amniotic fluid?

The authors state that women who are on antibiotics or have been on antibiotics were excluded from the study, but women who have cesarean sections get antibiotics.

Lines 203-207: in an already small sample size, 3 values were excluded for amniotic fluid CRP, 2 for maternal plasma CRP and 3 for maternal plasma IL-6. How did this affect the power of the analysis? Was this taken into account?

Lines 214-215: what is the author suggesting with the statement "some analytes did have a higher proportion of values below the LOD and should be interpreted with caution"? This adds doubt to the validity of the data.

Line 217: cord blood IL-6 was also excluded from the analysis since the proportion below the LOD for some conditions was greater than 50% and the true values of the measurements were unknown- was this also taken into account in the power analysis of the study? All cord blood IL-6 were excluded? There appears to be a lack of consistency in the assessment of the analytes.

Lines 246-248: the methodology used to calculate the power is not clear. What analyte was being used to calculate the power of the study? What is meant by a difference of at least 2.5 and 2.0? 2.5 and 2.0 are numbers referring to a difference of what numbers?

Results (lines 249-344)

Lines 252-254: may be more appropriate for the methods section

Lines 255-259: should be described in the methods as they describe the inclusion criteria.

Line 259: the primary objective does not need to be repeated in the results section.

Lines 270 - 281 and 333-334: There are statements are interspersed throughout the results section which are written more like a discussion than a result. No p-values or tables are referred to with the statements/interpretations.

Discussion:

Lines 365-368: it is not possible to so firmly conclude that fetal derived inflammatory mediators are likely signalers of parturition from this data. Without comparing analytes before and during labor in each woman in each of the 4 conditions, it is difficult to conclude that the differences in analytes noted in the fetal compartments are truly representative triggers of parturition.

Non-specific inflammatory markers may be elevated for a variety of reasons that cannot be controlled for. Furthermore, inflammatory markers may be significantly decreased in women undergoing cesarean sections who are receiving antibiotics-thereby weakening the argument significantly for the differences seen between term not in labor and term in labor women.

Line 407: please provide more detail regarding which confounders were "compared". Were these controlled for? If so how?

Line 405: Is this the initiation of the limitations section? Consider a new paragraph if this section discusses limitations. What other limitations exist in this kind of analysis? Do these analytes differ between races? Do these analytes differ in patients with higher or lower BMI? Were these controlled for or assessed statistically?
just cite the ranges for each entry. Also, since the samples range from 8 to 13, there is no basis for citing %s to nearest 0.1%. Instead, should round to nearest integer %.

Table 2: Again, the counts for each condition range from 8 to 13, so IQRs would be very unstable measures of variability. Should simply cite as ranges. If too cumbersome for this Table, could simply cite the medians in this table with supplemental table of either the ranges, or preferably, the entire data set for the interested reader.

Figs 1-3, lines 264-265: The samples are small and one cannot with any power or certainty distinguish normal vs non-normal distributions. It would make much more sense to uniformly employ non-parametric stats tests. Thus, any conclusions re: stats inference could not be questioned as a result of employing a test which had an assumption of a normal distribution. Two other issues arise from the small samples and the multiple comparisons. First, all NS findings potentially may have resulted from lack of stats power. Second, there were many comparisons done in each figure (1 overall ANOVA, 6 pairwise comparisons for each of 3 or 4 analytes, thus 21 or 28 comparisons). Based on multiple hypothesis testing, an inference threshold of p < .05 is inappropriately liberal and may have resulted in some spurious associations. Need to apply a stricter threshold.

lines 330-332, 369-371: Even using the inappropriate threshold of p < .05, this is not "marginal", rather the null hypothesis is not rejected.

EDITOR COMMENTS:

1. Thank you for your submission to Obstetrics & Gynecology. As you know, we rejected your paper on the first iteration as it seemed too "basic science" in focus for our journal. You have argued that it is really quite translational in nature and have made some substantial revisions to move it in that direction, to some effect. As such, we are requesting a revision but with major editing and changes needed to truly make it translational in nature. I recognize that the requested changes may be more than you are willing to do and that is a risk on my part.

See what you think with all of the reviewer comments and my own. Your work is fascinating and cutting edge and I hope we can work together to make it fit within the scope of our Journal. Cheers.

***In addition to the comments from the reviewers above, you are being sent a notated PDF that contains the Editor's specific comments. Please review and consider the comments in this file prior to submitting your revised manuscript. These comments should be included in your point-by-point response cover letter. The notated PDF is uploaded to this submission's record in Editorial Manager. If you cannot locate the file, contact Randi Zung and she will send it by email - rzung@greenjournal.org.***

- we avoid declarative titles. Please look at other titles in the Journal and edit to be consistent with that style.

- delete the highlighted and add a comma

- not sure what you mean by "uterotonic inflammatory markers". What is "uteronic" referring to? In clinical medicine, "uterotonic" typically refers to an agent that causes the uterus to contract. Is that your intent?

- 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.

- I don't think "theorize" here is the right verb. Perhaps "to inform a theory about the fetal and maternal inflammatory contributions"

- We have endorsed the ReVITALize terminology and definitions, available at https://www.acog.org/About-ACOG/ACOGDepartments/Patient-Safety-and-Quality-Improvement/reVITALize-Obstetric-Data-Definitions?IsMobileSet=false for ACOG members. Terminology has been updated so for instance, PROM now is "Prelabor rupture of the membranes". Please review reVITALize Please also make it clear what gestational ages your studied.

- Having the parenthesis after uric acid makes it seem that it relates only to uric acid. Recall that your readership in the GJ is a clinical readership and this is a translational paper so you will need to write it for a clinical audience. I would recommend rewriting this as the following, assuming it is consistent with your intention. We measured two damage-associated molecular pattern markers (HMGB1 and uric acid) and two acute phase response markers (IL-6 and C-Reactive Protein (CRP)).
- The Journal style doesn't use the virgule (/) except in numeric expressions. Please edit here and in all instances.

- Results were normalized, not the analytes

- I had to read your results a few times to be clear that you are reporting these by compartment and not by analyte. Can you instead report the values in terms of fetal side vs maternal side since your thesis is that the fetal side is the origin? The results as presented don't tell a story of what you are trying to describe. Recall your clinical readership here.

It's not clear what your p values refer to. What comparisons are the basis for these p values?

In both the abstract and the paper, please provide absolute numbers as well as which ever effect size you are reporting (if appropriate) + confidence intervals. P values may be omitted for space concerns. We strongly prefer CI's as they give more information about strength of association than do P values. By absolute values, I mean something like xx (outcome in exposed)/yy (outcome in unexposed) (zz%) (Effect size = ; 95% CI = .) An example might be: Outcome 1 was more common in the exposed than the unexposed 60%/20% (Effect size = 3; 95% CI 2.6-3.4). If you keep the p values, please report to no more than 3 decimal places.

- interventions for what?

- The introduction should be about 1 page length. Its currently 2. Please edit.

- You've lost me in the first 2 lines. As I read the first sentence, I understand you to mean that pregnancy is an inflammatory state. Can you state that more clearly? Perhaps something like "Pregnancy is an inflammatory state and a balance of pro- and anti-inflammatory factors is required for tissue remodeling [what tissue?] to facilitate feto-placental growth and development throughout gestation."

- This is vague. What senescent intrauterine tissues? Do you mean placental tissues? If so, state it. What do you mean "Reached their longevity"? If we already know that fetal organ maturation and intrauterine tissues are the source of inflammation that triggers labor, what does your paper add? What is the gap in knowledge?

- not sure what you mean "depending on the exposure". What exposure? Of what to what?

- facilitate or initiate labor? Facilitate makes it sound like to make labor go well.

- ambiguity implies that there is evidence supporting several points of view. Do you mean "incomplete knowledge"? I suggest moving the paragraph starting on line 82 to be the first paragraph of the introduction as it is the reason you are doing this work--its the clinical "hook" for your work.

- delete highlighted.

- since 1 of your groups wasn't in labor, this needs to be modified.

- to simplify (and shorten) ...."at the time of term and preterm delivery and with differing clinical scenarios."

- "may indicate"

- "specific to the obstetrical situation".

- "to theorize if there are different fetal and maternal inflammatory contributions..."

- spell out on first use.

- As noted by the reviewers and in particular the statistical reviewer, given that you have very small numbers of samples in your subgroups, it is important to do the following:
  1. Use non parametric analysis.
  2. Provide descriptive, not comparative, statistics. For instance, please delete the pair-wise comparisons

- "contribute to" is causal language. perhaps "that are associated with preterm birth"

- When you write that a study occurred between date 1 and date 2, it literally excludes those boundary dates. For instance, "This study was performed between Feb 2018 and Jan 2019" would mean it was performed from March 2018 to Dec 2018. Do you instead mean that the study was performed from date 1 to date 2? If so, please edit.

- All pregnancies resulted in singleton live births. note "Between" again in this sentence.

- This is a source of confusion. You have 4 groups of patients--in the parent paper, those with preterm births may have been the cases, but not in this paper. You are evaluating them all so they are all, technically, your
cases. Eliminate the use of the word "case". Preterm birth is typically defined as a birth at \( \leq 36 \) weeks 6 days, irrespective of etiology. In lines 109-111 I therefore have 2 questions. Why did you define this as \(< 36 \) weeks 0 days? Also, what you are describing is a preterm birth resulting from preterm labor. Presumably based on later information, these are all women in spontaneous preterm labor. Would you be willing to use that description for this group? Perhaps "one group of women were in spontaneous preterm labor, defined as delivery at \(< 36 \) week 0/7 weeks' gestation in the presence of regular uterine contractions ...."?

"Those with preterm prelabor rupture of the membranes (pPROM) were delivered at \(< 36 \) week 0/7 days [assuming you used this same gestational age definition] with membrane rupture diagnosed by..."

- please clarify: if you had patients for whom LMP and US dates differed, were they excluded so that you included only those with concordance?

- I am confused here. In line 104-105 you indicate that the samples were from preterm birth and following preterm prelabor rupture of membranes. You don't mention anything about term patients. Here you mention term patients. either your study samples included term patients (in which case that needs to be added to lines 104-105) or you can delete information here (lines 118-119) about term deliveries.

- I assume but you should clarify that labor was spontaneous in all cases and defined as above (line 110-111). Also, online 121 it should read "... AND not medical or obstetrical complications" shouldn't it?

- Would you consider calling this "elective repeat cesarean delivery" group instead of "term-not-in-labor"?

- it seems like later you have some preterm patients who delivered by cesarean. Why did you exclude intrapartum cesarean from one group but not the other? You also note in your results section that 36% of term patients delivered by CS.

Also, perhaps you could simplify lines 120-125 by saying something like "Women at term were included if they had no medical or obstetrical complications and delivered at \( \geq 37 \) week 0 days. The term group was divided into a term-labor group and a repeat elective cesarean delivery group. In the former, delivery followed the onset of labor, defined as the presence of uterine contractions occurring at least twice in 10 minutes. Women in the repeat cesarean group were delivered prior to the onset of labor or rupture of membranes.

- How was fluid collected for pPROM group?

- "during clinically-indicated phlebotomy" is clearer than "during diagnostic phlebotomy".

- before or after placental detachment? Please comment re: cleansing of cord. What is "free thawed"?

- Please shorten the description of the assays for the inflammatory markers by stating the kit type and maker in the paper, and reference details included in supplemental digital content.

- This section will need a lot of revision.

- Please state "Six results were excluded: three as they were considered to be non biologically plausible (amniotic fluid CRP and 2 maternal plasma CRP) and 3 for maternal plasma IL-6 [why did you exclude these last?]

- delete highlighted statement and then add "Amniotic fluid and cord blood CRP had 25% below LOD and cord blood IL-6 had 50% below it: these results should be interpreted with caution."

Spell out LOD throughout.

- Please just provide the name of the analyte rather than then type (ie, DAMP and cytokine). Instead, for "HMGB1, uric acid and IL-6 results were not normally distributed...." and then on line 225 "Results for CRP were normally distributed and ANOVA was determined...."

- this must be a SAS command. Please so state.

- please include the flow diagram you provided in the response to reviewer 1 from your original submission.

- here you introduce the idea of "late preterm". This is very confusing. You did not give a lower bound for EGA for inclusion of patients in your methods section, only an upper bound of \(< 36 \) week 0 days for the preterm group. This is a major issue as you have not presented your work to this point as being limited to late preterm patients (and in fact, you've excluded 1 of the 3 weeks included in late preterm definition of 34 week 0 d to 36 week 6 days).
- presumably you mean without clinical intrauterine infections or did you do cultures and placental pathology to exclude?

- should be in methods section (lines 256-259). Primary objection statement on line 259 should be in methods. I don't understand the "however" part of 260. What does that relate to? However what?

- I would think your primary objective was to measure concentrations of all 4 analytes in 3 biological compartments from pregnancies in different conditions—what you would fine (differences or similarities) would be the finding, not the objective. Then what do you mean by sentence starting on line 260 "However...."? Are there missing sentences? This seems like a non-sequitor from prior sentence.

- Numbers here are very small 7, 2, 6 and 6) so please avoid describing these characteristics as different ("women with pPROM had a higher frequency...", for instance) as you do not have statistical support for such a statement.

- These two sentences (lines 268-272) are contradictory. Rather than say in the first sentence that you DID not see a difference, state that you did see a difference in 2 analytes. This gets to my concerns that you are including women with labor (either term or preterm) with both vaginal and cesarean births and lumping them together. The paper would be MUCH cleaner if you had only vaginal births in the 3 groups and the term repeat CD not in labor group. This needs to be listed as a limitation.

- is this really THE most important thing you want to tell us? Its the lead sentence in your discussion. It seems to me that you want to tell us mechanistically what your paper is telling us. ...TNIL in group most quiescent; the inflammatory markers for women laboring (preterm and term) what do they do? what about the PPROMs?

- please provide 95% CI’s here.

- how does this analysis help? I would rather see the distribution by pregnancy grouping (TL, TNIL, PTB, PROM) for all analytes--figure 1, in essence but represent the 4 different analytes in the 3 compartments.

- As we are asking your to provide only descriptive statistics, your graphs will change somewhat (ie, p values edited out). As I try to paint a picture here of what is happening, the least "inflammatory" state is likely to be the woman who comes in with out labor and without PPROM for term scheduled CS. I recommend putting that group of results first in all of your whisker plots because every other group of patients in your study are likely to have inflammation beyond just what is present due to being pregnant. The TNIL group, with the eyeball test, does have the lowest or near lowest values for all 4 amniotic fluid analytes, corb blood HMGB1 and CRP, and maternal plasma IL6 and CRP. Not surprisingly, the Preterm group appears to have the highest values in general for most of your analytes in most of the compartments.

- I am very confused by the p value here. All of these values appear to overlap when including your whiskers. Same is really true for all of amniotic fluid values visually.

- limit all p values to 3 decimals throughout your paper.

- should this be PROM?

- should this be PROM?

- should there be a legend here for us to know that the white circles are TL, etc.

2. 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, as well as subsequent author queries. 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 response letter and subsequent email correspondence related to author queries. B. OPT-OUT: No, please do not publish my response letter and subsequent email correspondence related to author queries.

3. 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.
Any author agreement forms previously submitted will be superseded by the eCTA. During the resubmission process, you are welcome to remove these PDFs from EM. However, if you prefer, we can remove them for you after submission.

4. 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.

5. Have any of your figures been previously published in other sources?

Tables, figures, and supplemental digital content should be original. The use of borrowed material (eg, lengthy direct quotations, tables, figures, or videos) is discouraged, but should it be considered essential, written permission of the copyright holder must be obtained. Permission is also required for material that has been adapted or modified from another source. Both print and electronic (online) rights must be obtained from the holder of the copyright (often the publisher, not the author), and credit to the original source must be included in your manuscript. Many publishers now have online systems for submitting permissions request; please consult the publisher directly for more information.

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 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.

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, tables, boxes, figure legends, and print appendices) but exclude references.

8. Titles in Obstetrics & Gynecology are limited to 100 characters (including spaces). Do not structure the title as a declarative statement or a question. Introductory phrases such as "A study of..." or "Comprehensive investigations into..." or "A discussion of..." should be avoided in titles. Abbreviations, jargon, trade names, formulas, and obsolete terminology also should not be used in the title. Titles should include "A Randomized Controlled Trial," "A Meta-Analysis," or "A Systematic Review," as appropriate, in a subtitle. Otherwise, do not specify the type of manuscript in the title.

9. 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).

10. Provide a short title of no more than 45 characters (40 characters for case reports), including spaces, for use as a running foot.

11. Precis: Please do not use "indicates." Would you rephrase this to something else?

12. 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.

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

14. 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.

15. 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.

16. Figures

When you submit your revision, art saved in a digital format should accompany it. Please upload each figure as a separate file to Editorial Manager (do not embed the figure in your manuscript file).

If the figures were created using a statistical program (eg, STATA, SPSS, SAS), please submit PDF or EPS files generated directly from the statistical program.

17. 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 http://edmgr.ovid.com/acd/accounts/ifauth.htm.

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.

18. If you choose to revise your manuscript, please submit your revision via Editorial Manager for Obstetrics & Gynecology at http://ong.editorialmanager.com. It is essential that your cover letter list point-by-point the changes made in response to each criticism. Also, please save and submit your manuscript in a word processing format such as Microsoft Word.

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 Jun 21, 2019, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Nancy C. Chescheir, MD
Editor-in-Chief

2017 IMPACT FACTOR: 4.982
2017 IMPACT FACTOR RANKING: 5th out of 82 ob/gyn journals

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

Many thanks for reconsidering our manuscript for a second peer-review and allowing us to address critical concerns raised by you, the reviewers, and the statistical editor.

We have provided explanations to all queries on the following pages and have made substantial changes in the manuscript. We sincerely hope these changes are acceptable and our manuscript will be considered for publication.

A few points to highlight regarding our response:

1. This manuscript provides clinical relevance as to the source (fetal or maternal) of inflammation required to trigger parturition. Regulation of inflammation and or other labor triggering changes on the maternal side are the basis for current intervention or management of preterm labor subjects. Increasing trends in preterm birth and pPROM rates suggest that this approach has not been successful. This is the very first study to compare proinflammatory biomarkers from three distinct biological compartment from four different conditions to show that inflammatory accumulation on the fetal side is associated with term and preterm parturition. This data should highlight the importance of considering ‘fetus as a patient’ and direct (or design) intervention to minimize or control fetal inflammatory response.

2. As directed by you and the statistical editor, we have reanalyzed our data and described them as suggested. Figures are redrawn and descriptive stats are presented.

3. As requested by the editor and required by the journal, we have reduced both introduction and discussions. This has forced us to ignore some of the edits and explanations requested by reviewers. We hope that will not be counterproductive in the review process.

4. Due to substantial editing, some of the line numbers referenced by reviewers and editors may not match in the revised manuscript.
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.

Thank you,

Ramkumar Menon, PhD (Lead Author)
Associate Professor
REVIEWER #1:

1. Line 111: Why did you choose 36w0d rather than 36w6d?
   A. Samples for this study were drawn from a parent study (genetic and biomarker diversity in different racial/ethnic groups) that was funded by Thrasher Research Funds and March of Dimes USA. The scientific advisory committee and reviewer panel had given us a clear mandate that we use the cut off 360/7 to avoid any errors in LMP dating or US dating and misclassification errors of term and preterm births.

2. Lines 374-375: Did cost limit your study size or enrollment?
   A. Cost did not limit enrollment, but the availability of adequate volumes of three matching samples (amniotic fluid, maternal and cord plasma) from the same pregnancy was a limiting factor.

3. Line 402: Does not human data exist? Other mammals?
   A. To our knowledge, no animal model studies have been done using samples from three biological compartments. If the reviewer is referring to inflammation from the fetal side, multiple studies have been done on both animals and humans, but other than showing inflammation (cell level or a marker level) in individual tissues or fluids, no comprehensive studies have examined them to precisely determine a dominant response, i.e., fetal vs maternal. Using samples from same pregnancy and three different compartments, we can better propose the source of the effecter.

REVIEWER #2:

1. There have been numerous publications looking at the role of inflammatory mediators in parturition, both term and preterm. There are a handful of studies attempting to elucidate the source of the inflammatory "trigger" which leads to labor. Mendolsohn and colleagues looked at one mechanism by which the fetus may contribute to labor (J Steroid Biochem Mol Biol 2017 06;170:19-27), but their target differed from the target in the present study. Menon and colleagues have conducted a cross-section study of three inflammatory marker concentrations in three feto-maternal compartments in women from a previous study who had either pPROM, preterm labor, term labor, or term not in labor (cesarean deliveries). The identification of HMGB1 as higher in amniotic fluid in laboring patients (term labor and preterm labor) with no change in maternal plasma HMGB1 levels suggests that the fetus is the source of elevated HMGB1. Thus, this paper adds to a growing body of literature which works to identify the physiology surrounding normal labor. This is an necessary topic which may be highly sited.
   A. Thank you and we certainly appreciate this comment

2. The results section does require some review, as it is very difficult to follow. There may be a better way to present the analyses described, as well as to describe what is and is not statistically significant. One method may be to alter the description of each figure to explain. Similarly, the methods section regarding statistics is equally difficult to follow.
   A. In response to comments by the editor and statistical editor, the analyses will now primarily be descriptive and pairwise comparisons will be excluded. Subsequently, both the methods section and results have been edited substantially, Pages 7–14, and we have made a strong effort to improve the clarity of the results section.
REVIEWER #3:

1. **The methods section is much too long and needs to be edited down (8 pages long in its current format).**
   For example, it is not necessary to list the procedure for the HMGB1 ELISA kit—rather just state HMGB1 was analyzed according to the manufacturer's protocol.
   A. We agree with the reviewer and moved substantial portion of methods to supplemental material leaving only key sections, Pages 7–11.

2. **The statistical analysis of data is over 3 pages long, which again is much too long and needs to be edited down.**
   A. We have trimmed our statistical analysis section and additional details have been moved to supplemental section, Pages 9–11.

3. **The manuscript discusses how an accumulation of fetal inflammatory markers is associated with preterm birth. This has been published previously as far back as 1998 and I'm not sure if this descriptive study adds to this prior work:** R. Gomez, R. Romero, F. Ghezzi, et al. The fetal inflammatory response syndrome Am J Obstet Gynecol, 179 (1) (1998), pp. 194-202
   A. The reviewer is absolutely right that Dr. Romero’s group is the first one to report fetal inflammation as a mechanism of preterm birth. In fact, this author followed up Dr. Romero’s work to establish the fetal inflammatory response mediated by fetal membranes over the past 30 years. Data presented in this study are different as we are comparing classical (IL-6, other cytokines and CRP) and non-classical (DAMPs) fetal inflammatory mediators. DAMPs have recently been implicated in parturition and are thus a novel aspect of the study. We are showing that fetal inflammatory response is not necessarily mediated by makers as reported by Dr. Romero’s group. Further, we show that these markers may be compartment specific and condition specific as the distributions vary by labor status. In addition, we project that transition from non-labor to labor is manifested by fetal and not maternal inflammatory response and thus fetus may be the signaler (trigger) for parturition.

4. **I am not sure if this is an appropriate journal for this manuscript.**
   A. We believe that this topic is a very clinically relevant topic for the following reasons:
      1. Fetal inflammatory response and its compartment and marker specificity are unclear.
      2. Fetal inflammatory response and differential marker presence in 4 distinct conditions.
      3. Failure to diagnose and treat preterm labor and pPROM are partly due to this lack of knowledge.
      4. Novel mechanistic mediators of labor that can generate novel biomarker discovery strategies.
      5. Understanding how DAMPs are generated provides a whole new avenue of research to examine novel intervention targets.
      6. Biomarker discovery has been heavily focused on maternal plasma. However, in this study, we observed few changes in maternal plasma compared to fetal compartments.
REVIEWER #4:

Title
1. The title is appropriate for the review, and appropriate in length.

Summary
2. This is a cross-sectional study assessing whether inflammatory markers from amniotic fluid, cord blood, and maternal plasma were associated with parturition.

Abstract
3. Objective section: while the objective is appropriate it could be made clearer if authors were more specific. If the authors sought to determine whether mature fetal organs released inflammatory biomarkers that triggered parturition by examining biomarkers released in three feto-maternal compartments in women at term and preterm, it is not clear from the objective as written. This is not mentioned until the end of the introduction section, making it difficult for a reader to glean from the abstract.
   A. The abstract has word limitation, and we really can’t include too many details without compromising other sections of the abstract. We also like to point out that analytes measured are not coming from matured fetal organs although it could be one of the sources.

4. Methods section (lines 34-41): The abstract does not state that a parent study was used to conduct the cross-sectional analysis. The time period during which samples were taken, and the number required to be powered to see a significant difference between the groups was not stated. Why was the design unbalanced if this was taken from a larger cohort of patients? The analytes being compared are also non-specific and can differ per person at baseline.
   A. Please see our comment above. There are limitations to the extent of details we can include in the abstract. We have also stated in the manuscript is that restrictions to sample size is based on availability of 3 distinct samples from the same pregnancies. Although we may have plenty of subjects with matching samples, sample volume may not be adequate to perform all four assays.

5. Results section (lines 42-49): this section is unclear and confusing to the reader. It states that all amniotic fluid analytes and cord blood analytes (in line 43-44) differed across groups. The author then writes "in contrast, only maternal plasma IL-6 differed across groups". It is not clear whether the author is referring to the different labor groups or the different compartments being compared throughout this section. It may be easier to interpret the findings if the authors explained the findings as they relate to women who were term not in labor, term in labor, preterm in labor, and women with PPROM.
   A. We are sorry that the results are indeed confusing as the way it is written, and it is an oversight from our end. Thank you for pointing this out. We have now clarified this to be consistent and it reads as below (Page 4, Lines 44–48): “Fetal compartments (amniotic fluid and cord blood) showed higher HMGB1 in term labor vs. term not in labor and pPROM vs. PTB. Similarly, amniotic fluid IL-6 and cord blood CRP and uric acid (term vs term not in labor) and cord blood uric acid (pPROM vs PTB) were also higher. Only IL-6 in maternal plasma was higher in term labor compared to term not in labor.”
6. Line 47: The statement "Il-6 and uric acid were also elevated in the term labor compared to other conditions", which compartment is this referring to?

A. To avoid confusion in the results section, we have deleted this sentence.

7. Conclusion (lines 50-53): The authors state that HMGB1 and an increase in inflammatory markers on the fetal side instead of the maternal side. However, HMGB1 was not found to be increased in preterm labor per the results section. If the hypothesis is that mature fetal organs release biochemical mediators into the uterine environment, that is not clear from the results or the conclusion.

A. As mentioned above, these signals are not necessarily indications of fetal maturation. DAMPs release could be an after effect of biochemical signals produced by fetal organ maturation. However, our study did not investigate that nor have any evidence to claim that. We are merely stating that inflammatory accumulation is dominant on the fetal compartment than maternal biological side.

8. In addition, it is difficult not to associate an increase in inflammatory markers in PPROM due to some underlying infectious process that is not identified. Without comparing analytes before and during labor in each person per condition, it is difficult to conclude that the differences in analytes noted in the fetal compartments are truly representative of parturition.

A. This is an excellent point and we agree. However, as we stated in the methods, we have excluded all cases of infection in our study to avoid the potential confounding effects of infection. Comparing analytes before and during labor in each person per condition is almost impossible, as it will require both multiple amniocentesis and cordocentesis. This is partly why we used the next best approach as detailed in this manuscript. The reviewer is absolutely right, the fetal inflammatory response model needs to be mechanistically verified in animal models especially in response to risk conditions in pregnancy that can cause PTB and pPROM.

Introduction

9. (lines 58-96): This section of the manuscript is well written and provides good reasoning for the study question.

A. We thank the reviewer for this comment.

10. However, it is not clear why the particular biomarkers of HMGB-1, uric acid, IL-6 and CRP were chosen to be examined for this study.

A. Green journal is very strict about the word count in their introduction section that somewhat hinders us from elaborating. The editor has asked again to reduce the length of introduction to 1 page. Unfortunately, we could not include detailed introduction.

Materials and Methods

11. Lines (98-100): while the authors obtained their cohort from a parent study recruited from 2003-2011 to study racial disparities in biomarker profiles that contribute to preterm birth, the women selected for this study were predominantly white and only selected between the years 2008-2010, limiting the generalizability of the study. It is also not clear why these specific years were selected. How many patients were in the parent study and how was the subset of patients from the parent study selected? Was a flow diagram designed to show how subjects were included and excluded in the current study from the parent study?
A. The cohort was established in 2003 and existed until 2011. Twenty-three hundred subjects were enrolled during this period, and we added another ~1,500 subjects between 2008 and 2011. The parent study was funded for spontaneous preterm birth cases only, and additional funding was provided in 2008 to include pPROM. Therefore, we have restricted sample selection pool for this study for that period (2008–2011). In addition, we were using late PTB and pPROM, cases with no history of smoking and infection. At a rate of ~11% PTB in our cohort and 7% spontaneous PTB, we had ~166 subjects with spontaneous preterm birth, and 37.9% (41) of them had pPROM. Removing early PTBs, pPROMs, and infection cases (which are dominant in pPROM) and subjects with all 3 specimens with adequate sample volumes restricted our sample size. Exclusion criteria were stringent to avoid confounders that were explained above that have restricted our sample pool. We hope that this explanation will provide ample support for use of specific number of samples for this study.

![Diagram of recruitment period and sample selection process]

12. Line 111: Is there a reason for changing the definition of preterm delivery to <36 weeks 0 days from <37 weeks?
   A. Please see our comment to reviewer # 1

13. Lines 127-138: Was the same person collecting these samples? Could obtaining amniotic fluid after uterine incision or anesthesia and antibiotics received during a cesarean potentially change the biomarkers that could be present in amniotic fluid? The authors state that women who are on antibiotics or have been on antibiotics were excluded from the study, but women who have cesarean sections get antibiotics.
   A. CRP levels are used as a measure of effectiveness of antibiotics, so the reviewers’ concern is very valid. Hence, avoiding cases with infection in cases excluded this possibility. Women who are on antibiotics or have been on antibiotics were excluded from the study for term labor and not in labor subjects so as to avoid any impact of infection, signs of preterm labor and or other confounders among controls.
14. Lines 203-207: in an already small sample size, 3 values were excluded for amniotic fluid CRP, 2 for maternal plasma CRP and 3 for maternal plasma IL-6. How did this affect the power of the analysis? Was this taken into account?

A. We want to clarify that the power analysis was not conducted to determine the sample size of the study. A post-hoc analysis is conducted based on effect estimates and other parameters from the data. Thus, if the analysis was conducted excluding analytes, which it was, to derive estimates then the post hoc analysis considers these exclusions.

We would like to point out that the editor has requested that we change the analyses and report descriptive statistics. Additionally, we were initially cautious about providing a post-hoc power analysis in the first place. Post-hoc analyses calculate observed power, based on estimates from the data, thus are often argued as non-informative and do not indicate the true power of a study. In fact, Yuan and Maxwell have shown using simulations that observed power, when true power is small, is usually a biased indicator. Others have pointed out that post hoc power is highly dependent on \( P \) values and degrees of freedom, thus sample size does not influence the fact that \( P \) values can be converted to observed power. Given the revised direction of the analyses via comments from the editor and statistical editor, and valid arguments against post hoc analyses as informative indicators to the readers of true power, we have excluded the current text on post hoc analyses from the manuscript.


15. Lines 214-215: what is the author suggesting with the statement "some analytes did have a higher proportion of values below the LOD and should be interpreted with caution"? This adds doubt to the validity of the data.

Thank you for bringing this up as the LOD effect was neither interpreted nor stated right. We have revised this sentence to avoid any confusion. The revision was based on additional comments from the editor. We would like to clarify that the majority of analytes did not have large proportions below the LOD. Additionally, please note that LOD is a standard component of analytic assays and having values below the LOD is extremely common. This does not mean that data or assay is not valid. In fact, data below the LOD are often regarded as not clinically relevant because the cut-points for markers to make clinical decisions are usually well above the LOD (e.g., glucose levels). Data below the LOD indicates that the true value is somewhere between 0 and the LOD (the smallest value the assay can reliably detect). There are numerous approaches to handle data below the LOD, of which, our approach of replacing values with the LOD/sqrt 2 is preferred over exclusion or replacement with 0. Statistically speaking, large portions below the LOD can skew data but we are now employing non-parametric tests as requested by the statistical editor to accommodate non-normally distributed variables. There is numerous literature on the topic and due to the editor requesting reduced length of the methods/statistical analysis sections, an in-depth discussion of LOD is beyond the scope of this study. However, as this statement was confusing, the editor had specifically requested that we delete this statement and replace with the following (Page 10, Lines 154–157): “Amniotic fluid and cord blood CRP had 25% below the limit of detection and cord blood IL-6 had 50% below it. As a result, we have excluded cord blood IL-6 from statistical analyses and considered the potential influence of the limited of detection for amniotic fluid and cord blood CRP during the interpretation of our results.”
16. Line 217: cord blood IL-6 was also excluded from the analysis since the proportion below the LOD for some conditions was greater than 50% and the true values of the measurements were unknown- was this also taken into account in the power analysis of the study? All cord blood IL-6 were excluded? There appears to be a lack of consistency in the assessment of the analytes.

A. Please see comments above regarding how a post hoc power analysis is conducted.

Yes, all cord blood IL6 was excluded from the analysis. We do not agree that there is a lack of consistency in the assessment of the analytes. All analytes were examined for the proportion below the LOD. Cord blood IL6 was the only analyte to have a proportion below the LOD of ≥50%; thus, it was excluded across all groups (term labor, term not in labor, preterm birth, and PPROM). All values for all analytes which were determined to be below the LOD and could not be distinguished from true non-detectable levels were treated the same by imputing with the LOD by the square root of 2.

17. Lines 246-248: the methodology used to calculate the power is not clear. What analyte was being used to calculate the power of the study? What is meant by a difference of at least 2.5 and 2.0? 2.5 and 2.0 are numbers referring to a difference of what numbers?

A. Please see the comments above regarding the post hoc analysis and decision to exclude. Both editor and statistical editor also pointed out that power remains an issue given post hoc analyses are observed power that do not reflect true power and we have now deleted this section. Power analysis was not performed prior to the study as samples were gathered from a pool based select inclusion and exclusion criteria.

Results (lines 249-344)

18. Lines 252-254: may be more appropriate for the methods section

A. Moved to methods

19. Lines 255-259: should be described in the methods as they describe the inclusion criteria.

A. Moved to methods

20. Line 259: the primary objective does not need to be repeated in the results section.

A. Deleted

21. Lines 270 - 281 and 333-334: There are statements are interspersed throughout the results section which are written more like a discussion than a result. No p-values or tables are referred to with the statements/interpretations.

A. We agree, and this is in line with the format requested by the journal during previous review to provide a translational impact of data more understandable for clinical audience.

Discussion

22. Lines 365-368: it is not possible to so firmly conclude that fetal derived inflammatory mediators are likely signalers of parturition from this data. Without comparing analytes before and during labor in each woman in each of the 4 conditions, it is difficult to conclude that the differences in analytes noted in the fetal compartments are truly representative triggers of parturition. Non-specific inflammatory markers may be elevated for a variety of reasons that cannot be controlled for. Furthermore, inflammatory markers may be significantly decreased in women undergoing cesarean sections who
are receiving antibiotics—thereby weakening the argument significantly for the differences seen between term not in labor and term in labor women.

A. We agree with the reviewer. Epidemiologic studies, descriptive and sample studies like this will never prove functional impacts or causality as we need more in vitro and in situ animal models to prove them. As detailed above, repeated sample measures are impossible during human pregnancies, especially from fetal compartments (amniotic fluid and cord blood), and therefore, we used the best samples trios possible and avoid as many confounding factors as possible, selected markers that are specific and nonspecific to test their accumulation in cases (preterm) and controls (normal term). Based on the data and from ongoing studies (animal models: Sheller-Miller, S., Menon, R. Fetal Immune Cell Trafficking to Maternal Uterine Tissues in Animal Models of Term and Preterm Labor. March 2019: Society for Reproductive Investigation Annual Meeting: Oral Presentation; Manuscript submitted to Nature Communication), we believe that fetal inflammatory response promotes parturition in humans and maternal immune response and immune homeostasis maintains pregnancy. We agree with the reviewer that 4 biomarkers do not prove the concept; however, based on several recent reports and our own studies, we conceptualize that fetal inflammatory response tilts the immune balance to a pro-inflammatory state to promote labor. Please see our response to antibiotic use in the above sections.

We have stated this in our summary as follows (Page 16, Lines 294–297): “We conclude that fetal inflammatory marker accumulation is associated with human parturition at term and preterm. Although this descriptive study does not provide functional explanations or mechanistic evidence, we postulate that fetal-derived inflammatory signals are likely initial triggers of human parturition.”

23. Line 407: please provide more detail regarding which confounders were "compared". Were these controlled for? If so how?

A. Because the editor is requesting descriptive analyses, we are not conducting multivariable analyses of association. Thus, by nature of analysis, potential confounders are not adjusted for. An explanation of potential confounders was provided in the results in the initial submission, and we did not think it was necessary to repeat this information in the discussion section given the word limit. Thus, we had briefly acknowledged the potential for residual confounding and reminded readers that we did consider whether certain demographic and clinical variables were differed across groups and may be potential confounders (lines 406-413). The conditions for confounding are that the factor must be associated with exposure and outcome, must be unequally distributed among groups being compared and cannot be an intermediate in the causal pathways from exposure to outcome. Table 2 provided comparisons of distribution and frequency of potential confounders between groups, of which the vast majority were similar between groups. Unemployment (P = .029), income (P = .024), and cesarean delivery (P = .001) did significantly differ across conditions. However, when we explored whether the distribution of analytes (IL-6, CRP, HMGB1, and uric acid) in each condition differed by these maternal characteristics using the nonparametric Wilcoxon-Mann-Whitney test, the distribution did not significantly differ.

Again, given the request by the editor for this to be a descriptive analysis, we are not conducting multivariable models in this revised submission. We have revised the results sectional substantially and have rewritten the discussion to acknowledge the potential of residual confounding.

24. Line 405: Is this the initiation of the limitations section? Consider a new paragraph if this section discusses limitations. What other limitations exist in this kind of analysis? Do these analytes differ between races? Do these analytes differ in patients with higher or lower BMI? Were these controlled for or assessed statistically?
A. We have separated the two sections and included a paragraph detailing the limitations of this study (Pages 15–16). We have included other factors that are not yet examined, including BMI and fetal sex.

**STATISTICAL EDITOR’S COMMENTS:**

1. **Table 1:** The conditions each have small counts and SD would be very unstable measures of variability. Instead, should just cite the ranges for each entry. Also, since the samples range from 8 to 13, there is no basis for citing %s to nearest 0.1%. Instead, should round to nearest integer %.
   A. We thank the statistical editor for pointing this out. We have added ranges to the mean measurements. Per suggestion by the editor (please see the editor’s comments below), we have reformatted table 1 to include confidence intervals as well. We have also edited to round to the nearest integer %.

2. **Table 2:** Again, the counts for each condition range from 8 to 13, so IQRs would be very unstable measures of variability. Should simply cite as ranges. If too cumbersome for this Table, could simply cite the medians in this table with supplemental table of either the ranges, or preferrably, the entire data set for the interested reader.
   A. We have revised table 2 to provide medians and ranges rather than IQR.

3. **Figs 1-3, lines 264-265:** The samples are small and one cannot with any power or certainty distinguish normal vs non-normal distributions. It would make much more sense to uniformly employ non-parametric stats tests. Thus, any conclusions re: stats inference could not be questioned as a result of employing a test which had an assumption of a normal distribution.
   A. We thank the statistical editor for this suggestion. We have revised the manuscript to include non-parametric analyses across groups. This does not change the overall interpretation of our data. Per the editor’s request below, we have revised the manuscript to be descriptive.

4. **Two other issues arise from the small samples and the multiple comparisons.** First, all NS findings potentially may have resulted from lack of stats power. Second, there were many comparisons done in each figure (1 overall ANOVA, 6 pairwise comparisons for each of 3 or 4 analytes, thus 21 or 28 comparisons). Based on multiple hypothesis testing, an inference threshold of p < .05 is inappropriately liberal and may have resulted in some spurious associations. Need to apply a stricter threshold.
   A. We agree that lower power increases type II error and the possibility of accepting the null when the null should have been rejected. Small sample size and low power have been acknowledged in the discussion. As a standard approach, ANOVA was run for each of the 3–4 analytes; however, post-hoc analyses are only conducted if ANOVA rejects the null hypothesis. Analyses were not conducted for all analytes in all conditions. Post-hoc analyses correct for the type I error by maintaining a 0.05 family wise error rate rather than running individual t tests. Thus, for each post hoc analysis \( P=0.05 \) is appropriate to determine significance as error has already been accounted for. However, we acknowledge that multiple ANOVA and post-hoc analyses were conducted resulting in a total of 18 tests. As you are aware with 18 tests, the family wise error rate is \( \sim 0.60 \left[1-(1-\alpha)^{18}\right] \) which corresponds to a 60% chance that at least one test may be a false positive. With \( \alpha=0.05 \) we would expect \( \sim 1 \) test to be by chance (5% of 18).
   Regardless, as stated above, the editor has requested that we delete all pairwise comparisons and revise the analyses. Specifically, the editor would like us to present the analysis as primarily descriptive. We do believe that it is important for readers to provide a comparison across groups, using non-parametric tests.
as suggested by the statistical editor and the editor. We also believe that it is important to make some comparisons by labor group. Thus, for results that were significant across groups, we further compared term labor to term not in labor and pPROM vs Preterm birth. There are many arguments against correcting for type I error in certain situations throughout the epidemiologic and statistical literature, as you are aware. One being concern over the increase type II error and require larger sample sizes. Thus, we must weigh the chance of a false positive vs. further reducing our power. With 25 tests, our family wise error rate is ~0.72. Although the number of test is not excessively large, we have applied false discovery rate (FDR) to adjust $P$ values. In our opinion, this is more appropriate than Bonferroni correction given correlation among analytes. All results except the Kruskal-Wallis test of cord blood CRP across groups remained significant after FDR correction. This has been noted in the manuscript revisions.

References

5. lines 330-332, 369-371: Even using the inappropriate threshold of $p < .05$, this is not "marginal", rather the null hypothesis is not rejected.
   A. The use of “marginal” has been removed from the manuscript.

EDITOR’S COMMENTS:

1. Thank you for your submission to Obstetrics & Gynecology. As you know, we rejected your paper on the first iteration as it seemed too “basic science” in focus for our journal. You have argued that it is really quite translational in nature and have made some substantial revisions to move it in that direction, to some effect. As such, we are requesting a revision but with major editing and changes needed to truly make it translational in nature. I recognize that the requested changes may be more than you are willing to do and that is a risk on my part.
   A. Thank you, and we are more than happy to address the comments that are genuine and valid and will improve this work. I am a strong believer of fetal inflammatory response as a trigger for parturition, and not maternal. This is not just based on this work alone but multiple ongoing research work in my laboratory. We have undertaken substantial edits, reanalysis, rewriting of results to address several concerns that you have raised. Hope these changes are satisfactory.

2. Your work is fascinating and cutting edge and I hope we can work together to make it fit within the scope of our journal.
   A. I certainly appreciate this acknowledgement and Thank you very much for your continued support of my work.

3. We avoid declarative titles. Please look at other titles in the Journal and edit to be consistent with that style.
   A. We changed the title.
4. Delete the highlighted and add a comma
   A. Deleted and changed

5. not sure what you mean by "uterotonic inflammatory markers". What is "uteronic" referring to? In clinical medicine, "uterotonic" typically refers to an agent that causes the uterus to contract. Is that your intent?
   A. Yes, generally in basic science literature this terminology is used to refer cytokines and chemokines that have labor-inducing properties.

6. 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.
   A. We reviewed the instructions and have reduced our introduction, discussion sections, and number of references to comply with the journal’s formatting.

7. I don't think "theorize" here is the right verb. Perhaps "to inform a theory about the fetal and maternal inflammatory contributions"
   A. Changed

8. We have endorsed the ReVITALize terminology and definitions, available at
   https://nam03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.acog.org%2FAAboutACOG%2FACOGDepartments%2FPatient-Safety-and-Quality-Improvement%2FreVITALize-Obstetric-Data-Definitions%3FIIsMobileSet%3Dfalse&amp;data=02%7C01%7Cra2menon%40utmb.edu%7C769e6bccc00d443e9a1408d6e5f34e2c%7C7C7bef2f56d85db4526a72d31aee2546852%7C0%7C1%7C636949230484323741&amp;sdata=M3aUsRDdrrwSUAOJ%2BPHCcUTnqJFSX6YRXmM%2FxhrL9vU%3D&amp;reserved=0 for ACOG members. Terminology has been updated so for instance, PROM now is “Prelabor rupture of the membranes”. Please review reVITALize Please also make it clear what gestational ages your studied.
   We have now addressed terminologies and pPROM is changed to Prelabor rupture of the membranes

9. Having the parenthesis after uric acid makes it seem that it relates only to uric acid. Recall that your readership in the GJ is a clinical readership and this is a translational paper so you will need to write it for a clinical audience. I would recommend rewriting this as the following, assuming it is consistent with your intention. We measured two damage-associated molecular pattern markers (HMGB1 and uric acid) and two acute phase response markers (IL-6 and C-Reactive Protein (CRP)).
   A. Rewritten.

10. The Journal style doesn’t not use the virgule (/) except in numeric expressions. Please edit here and in all instances.
    A. All virgules were replaced.
11. Results were normalized, not the analytes  
   A. This has been deleted and is no longer required due to the use of non-parametric tests.

12. I had to read your results a few times to be clear that you are reporting these by compartment and not by analyte. Can you instead report the values in terms of fetal side vs maternal side since your thesis is that the fetal side is the origin? The results as presented don't tell a story of what you are trying to describe. Recall your clinical readership here.  
   A. We have rewritten results section while considering the suggested changes to the analyses as requested by the editor and statistical editor.

13. It's not clear what your p values refer to. What comparisons are the basis for these p values?  
   A. The \( P \) values were in regards to the ANOVA results, which is a test to determine if there are statistically significant differences between the means of two or more independent groups (term labor, term not in labor, preterm birth, and pPROM). Thus, a \( P \) value that results in rejecting the null hypothesis (eg, \( P < .05 \)) would indicate that there are at least two group means of the four groups (categorical variable consisting of: term labor, term not in labor, preterm and PPROM) that are significantly different from one another. An ANOVA cannot determine which groups actually differ. If ANOVA rejects the null hypothesis, then typically a post-hoc pairwise comparison is conducted to determine which group means actually differ.

However, below the editor has requested the following: (1) descriptive statistics only; (2) non-parametric tests and (3) exclusion of the pairwise comparisons. This will change the analyses and reporting in the abstract and results section.

14. In both the abstract and the paper, please provide absolute numbers as well as whichever effect size you are reporting (if appropriate) + Confidence intervals. \( P \) values may be omitted for space concerns. We strongly prefer CI's as they give more information about strength of association than do \( P \) values. By absolute values, I mean something like xx (outcome in exposed)/yy(outcome in unexposed) (zz%) (Effect size= ; 95% CI=). An example might be: Outcome 1 was more common in the exposed than the unexposed 60%/20% (Effect size=3;95% CI 2.6-3.4). If you keep the \( P \) values, please report to no more than 3 decimal places.  
   A. As the editor is requesting that descriptive analyses be the focus of the paper, we will not be conducting association tests. We have included CIs to accompany means and frequencies so that readers are able to see the variability in the data. Any \( P \) values reported have been changed to 3 decimal places.

15. interventions for what?  
   A. We have edited this section in the revised manuscript.

16. The introduction should be about 1 page length. Its currently 2. Please edit.  
   A. The intro is now limited to one page. We have ignored some requests from reviewer #4 who had asked to expand introduction to accommodate this request.

17. You've lost me in the first 2 lines. As I read the first sentence, I understand you to mean that pregnancy is an inflammatory state. Can you state that more clearly? Perhaps something like "Pregnancy is an inflammatory state and a balance of pro- and anti-inflammatory factors is required
for tissue remodeling [what tissue?] to facilitate feto-placental growth and development throughout gestation."
A. Changed; Page 6, Lines 58–59.

18. This is vague. What senescent intrauterine tissues? Do you mean placental tissues? If so, state it. What do you mean "Reached their longevity"? If we already know that fetal organ maturation and intrauterine tissues are the source of inflammation that triggers labor, what does your paper add? What is the gap in knowledge?
A. Sentence deleted

A. Clarified

20. Facilitate or initiate labor? Facilitate makes it sound like to make labor go well.
A. Changed to initiate

21. Ambiguity implies that there is evidence supporting several points of view. Do you mean "incomplete knowledge"? I suggest moving the paragraph starting on line 82 to be the first paragraph of the introduction as it is the reason you are doing this work--its the clinical "hook" for your work.
A. Moved and reworded as opening sentences in the introduction as suggested by the editor.

22. Delete highlighted.
A. Deleted

23. Since 1 of your groups wasn't in labor, this needs to be modified.
A. Modified

24. To simplify (and shorten) ...."at the time of term and preterm delivery and with differing clinical scenarios."
A. Changed

25. "may indicate"
A. Changed

26. "specific to the obstetrical situation".
A. Changed

27. "to theorize if there are different fetal and maternal inflammatory contributions..."
A. Changed

28. Spell out on first use.
A. Done
29. As noted by the reviewers and in particular the statistical reviewer, given that you have very small numbers of samples in your subgroups, it is important to do the following:

1. Use non-parametric analysis.

2. Provide descriptive, not comparative, statistics. For instance, please delete the pair-wise comparisons

A. We have revised our analysis and rewritten the results section. We are now focusing on descriptive statistics and have deleted the pair-wise comparisons that are examining every possible difference between groups. We want to clarify that a non-parametric analysis of analyze distributions across groups may not be considered “descriptive” but we believe it is important for readers, and suggested above, to at least provide these analyses. Specifically, this shows the reader that analyze distributions differ between two or more groups (term not in labor, term labor, preterm birth, and PPROM) in amniotic fluid and cord blood but that few changes were observed in maternal plasma. This is the primary objective of our study. By replacing ANOVA with non-parametric Kruskal-Wallis our overall interpretation of the data has not changed.

   We want to emphasize that by deleting pair-wise comparisons, we will not be able to statistically compare analytes between specific groups (e.g. Term not in labor vs. term labor). This is because Kruskal Wallis (non-parametric version of ANOVA) is an omnibus test statistic that cannot determine which groups have statistically different analyte distributions. To determine this a pairwise comparison would have to be conducted. We believe that we should make some non-parametric comparisons by laboring status for analytes that differed across groups. Specifically, term labor vs. term not in labor and PPROM vs. preterm labor. This is essentially to achieve the objectives of our paper. By making select comparisons this would also streamline the results and improve clarity as suggested by reviewers and editors. Additionally, in response to the statistical editor, we have corrected these results using false discovery rate to reduce type I error.

30. "contribute to" is causal language. perhaps "that are associated with preterm birth"

A. Changed

31. When you write that a study occurred between date 1 and date 2, it literally excludes those boundary dates. For instance, “This study was performed between Feb 2018 and Jan 2019” would mean it was performed from March 2018 to Dec 2018. Do you instead mean that the study was performed from date 1 to date 2? If so, please edit.

A. Edited

32. All pregnancies resulted in singleton live births. note "Between" again in this sentence.

A. Changed

33. This is a source of confusion. You have 4 groups of patients—in the parent paper, those with preterm births may have been the cases, but not in this paper. You are evaluating them all so they are all, technically, your cases. Eliminate the use of the word "case". Preterm birth is typically defined as a birth at \( \leq 36 \) weeks 6 days, irrespective of etiology. In lines 109-111 I therefore have 2 questions. Why did you define this as \(<36\) weeks 0 days? Also, what you are describing is a preterm birth resulting from preterm labor. Presumably based on later information, these are all women in spontaneous preterm labor. Would you be willing to use that description for this group? Perhaps "one
group of women were in spontaneous preterm labor, defined as delivery at < 36 week 0/7 weeks' gestation in the presence of regular uterine contractions ....."?
A. Explained above

34. "Those with preterm prelabor rupture of the membranes (pPROM) were delivered at < 36 week 0/7 days [assuming you used this same gestational age definition] with membrane rupture diagnosed by...
A. Clarified

35. Please clarify: if you had patients for whom LMP and US dates differed, were they excluded so that you included only those with concordance?
A. Clarified

36. I am confused here. In line 104-105 you indicate that the samples were from preterm birth and following preterm prelabor rupture of membranes. You don't mention anything about term patients. Here you mention term patients, either your study samples included term patients (in which case that needs to be added to lines 104-105) or you can delete information here (lines 118-119) about term deliveries.
A. Clarified

37. I assume but you should clarify that labor was spontaneous in all cases and defined as above (line 110-111).
A. Changed

38. Also, online 121 it should read "... AND not medical or obstetrical complications" shouldn't it?
A. This is not clear to us and left it as is.

39. Would you consider calling this "elective repeat cesarean delivery" group instead of "term-not-in-labor"?
A. This is a standard terminology we and others have been using to describe not in labor groups. As this paper will also be read by non-clinicians, I would prefer to use a term that has been frequently used in the literature.

40. It seems like later you have some preterm patients who delivered by cesarean. Why did you exclude intrapartum cesarean from one group but not the other? You also note in your results section that 36% of term patients delivered by CS.
A. Term labor cesareans were excluded since forbag-hindbag compartmentalization can cause variation in analyte concentrations (MacDonald PC and Casey ML studies of the 90s). This can confound data. So to be on the safe side and to present cleanest data, we have excluded them.

41. Also, perhaps you could simplify lines 120-125 by saying something like "Women at term were included if they had no medical or obstetrical complications and delivered at >=37 week 0 days. The term group was divided into a term-labor group and a repeat elective cesarean delivery group. In the former, delivery followed the onset of labor, defined as the presence of uterine contractions occurring
at least twice in 10 minutes. Women in the repeat cesarean group were delivered prior to the onset of labor or rupture of membranes.
A. Changed

42. "during clinically-indicated phlebotomy" is clearer than "during diagnostic phlebotomy".
A. Changed

43. - before or after placental detachment? Please comment re: cleansing of cord. What is "free thawed"?
A. Clarified as before placental detachment. Changed to read “freeze thawed” (Page 9, Line 130).

44. Please shorten the description of the assays for the inflammatory markers by stating the kit type and maker in the paper, and reference details included in supplemental digital content.
A. Shortened and moved as supplemental material.

45. This section will need a lot of revision.
A. Revised and rewritten according to the suggested changes in the statistical analysis by the editors.

46. Please state "Six results were excluded: three as they were considered to be non biologically plausible (amniotic fluid CRP and 2 maternal plasma CRP) asnd 3 for maternal plasma IL-6 [why did you exclude these last?]
A. We thank you for pointing out that this state is confusing as written. All were excluded because they were determined to be non-biologically plausible. We have revised the sentence.

47. delete highlighted statement and then add "Amniotic fluid and cord blood CRP had 25% below LOD and cord blood IL-6 had 50% below it: these results should be interpreted with caution."
A. Changed. We want to clarify that values below the LOD are not abnormal and that we reasonably excluded IL6 that may be problematic for analysis (proportion LOD >50%). Additionally, only a few analytes had higher proportions above the LOD (e.g. 25%), thus, we do not want to make an inappropriate suggestion the data is not valid. We suggest the following edit to the editor’s revision (Page 10, Lines 154–157): “Amniotic fluid and cord blood CRP had 25% below the limit of detection and cord blood IL-6 had 50% below it. As a result, we have excluded cord blood IL6 from statistical analyses and considered the potential influence of the limited of detection for amniotic fluid and cord blood CRP during the interpretation of our results.”

48. Spell out LOD throughout.
A. Spelled out

49. Please just provide the name of the analyte rather than then type (ie, DAMP and cytokine). Instead, for "HMGB1, uric acid and IL-6 results were not normally distributed...." and then on line 225 "Results for CRP were normally distributed and ANOVA was determined...."
A. As requested non-parametric analyses are being used and thus, normalization is not needed nor is ANOVA, this sentence has been deleted.
50. This must be a SAS command. Please so state.
   A. Yes, but has been deleted as it is no longer relevant. See above.

51. Please include the flow diagram you provided in the response to reviewer 1 from your original submission.
   A. The flow diagram included was for original study cohort as requested by the reviewer. As stated in response to reviewer here, we have included a flow diagram for the time period for which these samples were ascertained. The original flow diagram is published elsewhere, it is not appropriate to repeat that information in this work. If you feel strongly that it should be included, we are happy to include the new figure that won’t conflict with copyright issues and will be more appropriate for this study.

52. Presumably you mean without clinical intrauterine infections or did you do cultures and placental pathology to exclude?
   A. Explained

53. Should be in methods section (lines 256-259). Primary objection statement on line 259 should be in methods. I don't understand the "however" part of 260. What does that relate to? However what?
   A. Moved and clarified

54. I would think your primary objective was to measure concentrations of all 4 analytes in all 3 biological compartments from pregnancies in different conditions—what you would find (differences or similarities) would be the finding, not the objective. Then what do you mean by sentence starting on line 260 "However...." ? Are there missing sentences? This seems like a non-sequitor from prior sentence.
   A. Clarified

55. Numbers here are very small 7, 2, 6 and 6) so please avoid describing these characteristics as different ("women with pPROM had a higher frequency...", for instance) as you do not have statistical support for such a statement.
   A. Although the numbers are small, data show significance, and thus, were not affected by type II error and warrant explanation. However, we have accepted the editor’s suggestions. This section has been revised in order to provide descriptive statistics only.

56. These two sentences (lines 268-272) are contradictory. Rather than say in the first sentence that you DID not see a difference, state that you did see a difference in 2 analytes. This gets to my concerns that you are including women with labor (either term or preterm) with both vaginal and cesarean births and lumping them together. The paper would be MUCH cleaner if you had only vaginal births in the 3 groups and the term repeat CD not in labor group. This needs to be listed as a limitation.
   A. We have revised this section. As noted above, the analysis has been changed to be descriptive. However, this is an example of why we may need to make some comparisons between groups to address reviewer and editors concerns. As a compromise, rather than providing extensive comparisons we will just acknowledge the limitations of potential confounding and inclusion of both cesarean and vaginal births while providing a few counter-points based on our data. Please see the discussion.
Additionally, we have further considered this request. Only examining vaginal births would result in 7 term labors, 8 preterm labors and 3 pPROMs. This will reduce the accuracy of our descriptive analyses such as the distributional statistics as pointed out by the statistical editor. It is also unlikely to change our results. For example, if we exclude CS (except obviously for the term not in labor group), according to the non-parametric Kruskal-Wallis test our results essentially remain unchanged. Amniotic fluid HMGB1 ($P<.0001$), IL-6 ($P=.0002$) and uric acid (0.012) distributions still significantly differ between two or more groups. As do cord blood HMGB1 ($P=.0007$) and maternal plasma IL6 ($P=.0122$).

An explanation has been provided for the vaginal vs cesarean delivery biomarker differentials. Sampling is critical only when hindbag and forbag are separated and that too for amniotic fluid samples only.

57. edit the results now for descriptive data only.
   A. The results have been edited. However, please note that above non-parametric comparisons were requested above.

58. Is this really THE most important thing you want to tell us? Its the lead sentence in your discussion. It seems to me that you want to tell us mechanistically what your paper is telling us. ...TNIL in group most quiescent; the inflammatory markers for women laboring (preterm and term) what do they do? what about the PPROMs?
   A. We have edited the discussion paragraph.

59. Please provide 95% CI's here.
   A. This comment refers to table 1 and the $P$ values that were primary calculated using Fisher’s exact tests. This simply determines if the proportions for one variable are different among values of another variable. With Fisher’s exact you could use a 2X2 table to create effect estimates (OR and RR) and CIs. As conditions (term labor, term not in labor, preterm birth and pPROM) is a 4-level variable this actually is not a 2X2 table. We could select a comparison group and create separate variables (eg, TL vs TNIL; PTB vs TNIL, etc.) which would result in numerous statistical comparisons. The statistical editor has pointed out numerous comparisons as a concern. This would also not be consistent with descriptive statistics that were requested. Keeping with the theme of “descriptive statistics” we will report frequencies and CIs (or mean CIs). This will provide readers with information regarding the variability in the data, of which, they can make inferences regarding comparisons.

60. How does this analysis help? I would rather see the distribution by pregnancy grouping (TL, TNIL, PTB, PROM) for all analytes--figure 1, in essence but represent the 4 different analytes in the 3 compartments.
   A. We have excluded these additional correlation analyses from the manuscript. Additionally, we have revised the figures so that each figure represents an analyte. For each analyte, the distribution within term labor, term not in labor, preterm birth, and pPROM are displayed for amniotic fluid, cord blood, and maternal plasma.

61. As we are asking you to provide only descriptive statistics, your graphs will change somewhat (ie, p values edited out). As I try to paint a picture here of what is happening, the least "inflammatory" state is likely to be the woman who comes in with out labor and without PPROM for term scheduled CS. I recommend putting that group of results first in all of your whisker plots because every other group of patients in your study are likely to have inflammation beyond just what is present due to being pregnant. The TNIL group, with the eyeball test, does have the lowest or near lowest values for all 4
amniotic fluid analytes, cord blood HMGB1 and CRP, and maternal plasma IL6 and CRP. Not surprisingly, the Preterm group appears to have the highest values in general for most of your analytes in most of the compartments.

A. We have removed $P$ values and reordered the groups so that TNIL is presented first. Although please note that non-parametric tests were still requested and this would be equivalent to the $P$ values that are currently in the figures.

62. I am very confused by the p value here. All of these values appear to overlap when including your whiskers.

A. The $P$ values are direct output from SAS. Visualizing of a boxplot cannot definitively determine if there is a statistical difference between groups. Typically, box and whisker plots are interpreted as follows: when the median of one group is outside of the box (IQR) of another group box (excluding whiskers), this simply indicates that there is likely a difference between two groups, but many factors play a role. Whiskers are outside of the box and are representations of the range of data. As pointed out by the statistical editor, IQR can be influenced by sample size. Still, if you look at amniotic fluid HMGB1 for example, the median for TNIL is outside every other group box, thus, this would indicate a likely difference. Even for IL6, which this comment is referring to, the median for TNIL is outside the box for TL, which is likely a significant difference. Keep in mind, the $P$ values are from output from ANOVA. As explained above, ANOVA in an omnibus test and a significant $P$ value only tells us that the analyte mean differs between two or more groups. This does not indicate which groups differ. Pairwise comparisons are needed to determine which groups may significantly differ.

63. Same is really true for all of amniotic fluid values visually.

A. Please see the statement above.

64. Limit all p values to 3 decimals throughout your paper.

A. This has been revised.

65. Should there be a legend here for us to know that the white circles are TL, etc.

A. The legends were to the right of each graph but per the comment above, these analyses have been deleted from the manuscript.

We hope that these extensive changes, including reanalysis of data according to guidelines and instructions provided by the reviewers, editor, and statistical editor, are satisfactory. Once again, we thank the reviewers and editors for their incredible constructive comments and critiques with this manuscript.

Sincerely,

Ram and Brandie