OBSTETRICS & GYNECOLOGY

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

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

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Date: Apr 23, 2021
To: "Jeffery Goldstein" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-21-822

RE: Manuscript Number ONG-21-822

SARS-CoV-2 vaccination in pregnancy: measures of immunity and placental histopathology

Dear Dr. Goldstein:

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 as a Research Letter due in 1 week for fast-track publication.

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 7 days from the date of this letter. If we have not heard from you by Apr 30, 2021, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1:

The authors study the placental immuno-histopathology of women with and without vaccinations for SARS Co-V-2

1. Line 89: placentas were evaluated under an "equivalent research protocol"- is that for this study or another study?
2. Line91: therefore, not all placentas in these two cohorts were studied? Or, the remainder were collected as part of clinical care. Please clarify this distinction.
3. Line 95- the word "parsed" : perhaps it might offer more clarity to use the word "analyzed" as a more common substitution?
4. Line 148: with respect with your limitations is the lack of certainty that the SARS Co-V-2 negative women cohort were true negatives given your assessment of them.

Reviewer #2:

The authors present placenta examination results in women who did and did not receive the covid mRNA vaccine in pregnancy to assess if vaccination in pregnancy is associated with placental lesions such as inflammation. I have several questions for the authors.

1. the premise for the study is interesting, but not intuitive to this Reviewer. Has this been studied with other vaccines in pregnancy either showing there were placental lesions with vaccination, or using placental pathology as a way to demonstrate safety of another vaccine?
2. to be clear, the prospective cohort were the vaccinated patients, but the controls were retrospective cases? or was there a prospective study already ongoing prior to the vaccination roll out?
3. did patients provide consent for this?
4. Methods, line 99. which aforementioned lesions? (i dont see where you list which ones you were examining in this study)
5. if the power analysis assumed a 1:2 ratio and yielded 50 cases and 100 controls, why were there ultimately 71 cases and 107 controls in the study?

6. how were controls selected? presumably, there were more than 107 women who delivered without covid or covid vaccine over a 12-month period. were they selected randomly?
7. combining questions 2,3,5, and 6 into one overarching question: exactly when and how were patients (or placentas) recruited for this study? it is unclear from the methods and it is hard to see how this could be called a prospective study when the controls were collected many months before anyone got vaccinated.

Reviewer #3:

Abstract: Need to include a concise summary of the quantitative findings that would support the conclusions. Also, need to clarify that the N = 71 and 107 were greater than the samples with measured antibodies or with placental pathology. Need to address the missing data in main text as a potential source of selection bias. Need to cite the baseline characteristics of the analyzed vs non-analyzed samples.

lines 97-105, Table 1: There are two issues with the sample size calculation. First, there were 5 primary outcomes, so the choice of $p < .05$ as the alpha is inappropriate; it does not account for multiple hypothesis testing. Second, the Authors need to supply a reference or other rationale for using a rate of abnormal findings 3x that of the control as adequate to demonstrate an equivalence of controls vs vaccinated.

lines 91, Table 1: Need to clarify the number of placentas included in the study and in Table 1. Why were placentas not in research protocol included in the analysis?

Fig 1: Legend should include concise summary of the stats comparisons of the two groups.

Table 1: Should make this into two tables, one with baseline characteristics and the second with outcomes. Need to clearly separate the primary outcomes vs the rest. The vaccinated group had N = 71, so all %s in that column should be rounded to nearest integer %, not cited to 0.1% precision. Also, if not all women had antibody titers, then should identify the samples represented by that row.

lines 102-105: The comparisons of placental lesions involved many with small counts (< 5) in one or both groups. Thus, chi-square is an inappropriate test, should have used Fisher's exact test. Also, many of those comparisons had such small counts of abnormal findings that including another covariate (gestational age at delivery) results in over fitting of the model. Should simply have used Fisher's test to compare the counts.

EDITOR COMMENTS:

Thank you for submitting your work to Obstetrics and Gynecology. If you would like to submit a revision, please format as a research letter and focus on the descriptive findings given the limitations of the sample size.

EDITORIAL OFFICE COMMENTS:

1. The Editors of Obstetrics & Gynecology are seeking to increase transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:
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3. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

4. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Research Letters articles should not exceed 600 words. Stated word limits include the title page, précis, abstract, text, tables, boxes, and figure legends, but exclude references.

5. 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).
* If your manuscript was uploaded to a preprint server prior to submitting your manuscript to Obstetrics & Gynecology, add the following statement to your title page: "Before submission to Obstetrics & Gynecology, this article was posted to a preprint server at: [URL]."

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

7. Provide a précis on the second page, for use in the Table of Contents. The précis is a single sentence of no more than 25 words that states the conclusion(s) of the report (ie, the bottom line). The précis should be similar to the abstract's conclusion. Do not use commercial names, abbreviations, or acronyms in the précis. Please avoid phrases like "This paper presents" or "This case presents."

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

9. 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.

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

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

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

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

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Figures should be saved as high-resolution TIFF files. The minimum requirements for resolution are 300 dpi for color or black and white photographs, and 600 dpi for images containing a photograph with text labeling or thin lines.

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If you choose to revise your manuscript, please submit your revision through Editorial Manager at http://ong.editorialmanager.com. Your manuscript should be uploaded in a word processing format such as Microsoft Word. Your revision's cover letter should include the following:

* A confirmation that you have read the Instructions for Authors (http://edmgr.ovid.com/ong/accounts/authors.pdf), and
* A point-by-point response to each of the received comments in this letter. Do not omit your responses to the Editorial Office or Editors' comments.

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

Again, your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Apr 30, 2021, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Torri D. Metz, MD
Associate Editor, Obstetrics

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

[redacted]

April 23, 2021

Torri D. Metz, MD
Associate Professor
Department of Obstetrics and Gynecology
University of Utah School of Medicine

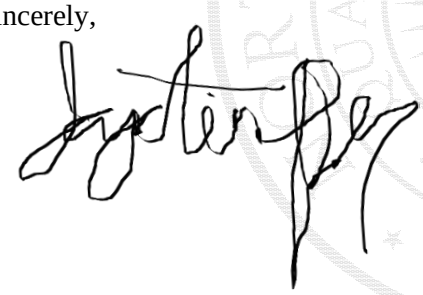
Dear Dr. Metz,

Thank you for your review of our manuscript and invitation to resubmit as a research letter. We submit for your consideration the attached revised and reformatted research letter, “SARS-CoV-2 vaccination in pregnancy: measures of immunity and placental histopathology”

We have read the instructions for submission.

We are grateful for the thoughtful reviews and are pleased to have our responses to reviewers published. Those responses are below.

Thank you for your consideration.
Sincerely,



Jeffery A. Goldstein MD, PhD
Assistant Professor
Department of Pathology
Northwestern University Feinberg School of Medicine

[redacted]

Reviewer #1:

1.1 Line 89: placentas were evaluated under an "equivalent research protocol"- is that for this study or another study?

We allude to the common criticism of placental pathology papers that <25% of placentas are submitted for histopathology, limiting generalizability.[1,2] Our study includes both placentas from vaccinated and control patients where clinical exam was requested (reports abstracted from the laboratory information system) and those where clinical exams were not requested but placenta was collected for the purposes of this study. We have rewritten the *Methods* to better clarify this matter.

1.2 Line 91: therefore, not all placentas in these two cohorts were studied? Or, the remainder were collected as part of clinical care. Please clarify this distinction.

The remainder were collected for clinical care. See response 1.1.

1.3 Line 95- the word "parsed" : perhaps it might offer more clarity to use the word "analyzed" as a more common substitution?

We have replaced a narrative of this method with a citation to its original use.[3]

1.4 Line 148: with respect with your limitations is the lack of certainty that the SARS Co-V-2 negative women cohort were true negatives given your assessment of them.

Controls were defined as SARS-CoV-2 PCR negative, IgG and IgM negative at delivery, vaccine negative, which is now more explicitly stated in the *Methods*.

Reviewer #2:

2.1 the premise for the study is interesting, but not intuitive to this Reviewer. Has this been studied with other vaccines in pregnancy either showing there were placental lesions with vaccination, or using placental pathology as a way to demonstrate safety of another vaccine?

We are unaware of published work examining associations of placental pathology with vaccination.

COVID-19 has shone light on a few previously ignored areas. For example, the largest study in 2009 H1N1 influenza infection has 15 cases, no explicit control group, and was published in 2014.[4] Conversely, placental pathology in COVID-19 is a rich and contentious subfield, with the first US publication less than 4 months after the first US case.[5]

Some medications, when used in pregnancy, are associated with placental changes, including accelerated villous maturation with glucocorticoids,[6] abnormal placental shape with HIV protease inhibitors,[7] and chronic villitis or intervillositis after antineoplastic chemotherapy.[8] In addition, as described in the introduction of this manuscript, the novel mRNA vaccines induce an immune response via direct mRNA activation of TLR3, a process which has been associated in some mouse models with placental and fetal pathology.

With this background, there is scientific rational for potential placental pathology associated with COVID-19 vaccination, which motivates our study.

2.2 to be clear, the prospective cohort were the vaccinated patients, but the controls were retrospective cases? or was there a prospective study already ongoing prior to the vaccination roll out?

This study includes patients from our ongoing COVID-19 study, initiated prior to the vaccine roll-out. The prospective study began prior to vaccination roll-out but continued and was expanded to include vaccinated patients. Controls have been collected throughout the pandemic and continue to date. This has been clarified in the methods.

2.3 did patients provide consent for this?

The study operated under waiver of consent.

2.4 Methods, line 99. which aforementioned lesions? (i dont see where you list which ones you were examining in this study)

We have removed the comment. A formal list of target lesions is in the *Table*.

2.5 if the power analysis assumed a 1:2 ratio and yielded 50 cases and 100 controls, why were there ultimately 71 cases and 107 controls in the study?

We continued to accrue patients with vaccination and controls during the analysis (50->71) and revision (71->84) stages. We feel it is preferrable to publish with the most up-to-date data available. See comment 3.2 for further discussion of power and sample size.

2.6. how were controls selected? presumably, there were more than 107 women who delivered without covid or covid vaccine over a 12-month period. were they selected randomly?

Controls were derived from a pool of controls from another arm of the study evaluating placental findings in COVID-19 disease. Controls were SARS-CoV-2 PCR negative and vaccine negative (after rollout) and matched to COVID-19 patients delivering within 14 days at the same gestational age. Among potential controls, the selection was stochastic. This has been clarified in the *Methods*.

2.7. combining questions 2,3,5, and 6 into one overarching question: exactly when and how were patients (or placentas) recruited for this study? it is unclear from the methods and it is hard to see how this could be called a prospective study when the controls were collected many months before anyone got vaccinated.

Patients were identified for inclusion at the time of delivery. Samples and EHR data were collected under waiver of consent. The study is prospective in that samples were collected after initiation of the study. The manuscript has been edited to remove any description of the study as prospective to enhance clarity.

Reviewer #3:

3.1 Abstract: Need to include a concise summary of the quantitative findings that would support the conclusions. Also, need to clarify that the N = 71 and 107 were greater than the samples with measured antibodies or with placental pathology. Need to address the missing data in main text as

a potential source of selection bias. Need to cite the baseline characteristics of the analyzed vs non-analyzed samples.

The abstract has been deleted due to reformatting. See 1.1, 1.2, 2.2, 2.3, 2.5, 2.6 and *Methods* for discussion of missing or un-analyzed data. All 116 controls had negative anti-SARS-CoV-2 antibodies and available placental pathology reports. 52/84 patients with vaccination had antibody testing. We feel it is appropriate to report the placental findings from the whole group as the exposure at issue is vaccination *per se*, regardless of whether immunity developed.

3.2 lines 97-105, Table 1: There are two issues with the sample size calculation. First, there were 5 primary outcomes, so the choice of p < .05 as the alpha is inappropriate; it does not account for multiple hypothesis testing. Second, the Authors need to supply a reference or other rationale for using a rate of abnormal findings 3x that of the control as adequate to demonstrate an equivalence of controls vs vaccinated.

The original sample size calculation was driven by evaluating decidual arteriopathy, as we feel it has the strongest association with COVID-19 disease in humans and TLR3 activation in mice.[3,9] COVID-19 is associated with a 2-4 fold increased risk of decidual arteriopathy (in our data). 2009 H1N1 influenza and antineoplastic chemotherapy are associated with estimated 3-fold increases in the risk of chronic villitis. [8,10] Therefore, we felt a 3 fold increased risk was an appropriate threshold for this preliminary analysis.

On reflection, we agree the *a priori* calculation is a poor fit for the study as reported. We therefore report a *post hoc* power calculation, demonstrating at least 80% power to identify at least a 2.5-fold increased risk of any lesion with a baseline prevalence >=10% and a 3-fold increased risk of any lesion with a baseline prevalence of >=7%

See wurty 2.5 for further discussion.

3.3 lines 91, Table 1: Need to clarify the number of placentas included in the study and in Table 1. Why were placentas not in research protocol included in the analysis?

Placentas from included patients that were submitted for clinical examination were included. See 1.1 and 1.2.

3.4 Fig 1: Legend should include concise summary of the stats comparisons of the two groups.

Statistical comparisons are presented in the *Table*

3.5 Table 1: Should make this into two tables, one with baseline characteristics and the second with outcomes. Need to clearly separate the primary outcomes vs the rest. The vaccinated group had N = 71, so all %s in that column should be rounded to nearest integer %, not cited to 0.1% precision. Also, if not all women had antibody titers, then should identify the samples represented by that row.

Due to space limitations, we are limited to 1 figure and 1 table. As suggested, %s are changed to the nearest integer. All controls and 52/84 vaccinated patients had antibody levels measured. The n-sizes are listed in the left hand column of the table.

3.6 lines 102-105: The comparisons of placental lesions involved many with small counts (< 5) in one or both groups. Thus, chi-square is an inappropriate test, should have used Fisher's exact test. Also, many of those comparisons had such small counts of abnormal findings that including another covariate (gestational age at delivery) results in over fitting of the model. Should simply have used Fisher's test to compare the counts.

We did, in fact, use Fisher exact test to compare categorical demographic features and logistic regression was used for placental lesions (*Methods*). Chi-square was referenced in error. Gestational age is the single most important risk factor for adverse pregnancy outcomes and most placental lesions. Given the relative homogeneity of gestational ages at delivery in this study, it has relatively little impact on the interpretation of this study. A version of the analysis using Fisher's exact test is given below:

Diagnosis	P (Fisher)	OR (Fisher)	Neither vaccination nor diagnosis	Vaccination without diagnosis	Diagnosis without vaccination	Vaccination and diagnosis
Decidual arteropathy	0.651237	0.766917	102	76	14	8
Fetal vascular malperfusion	1	0.85443	108	79	8	5
Low grade chronic villitis	0.339204	1.606607	107	74	9	10
High grade chronic villitis	0.053909	0.3125	100	80	16	4
Chronic intervillositis	0.510352	0	114	84	2	0

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10. Meijer WJ, Wensing AMJ, Bruinse HW, Nikkels PGJ. High Rate of Chronic Villitis in Placentas of Pregnancies Complicated by Influenza A/H1N1 Infection. *Infectious Diseases in Obstetrics and Gynecology*. 2014;2014:1-5. doi:10.1155/2014/768380