OBSTETRICS & GYNECOLOGY

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

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

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

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

**Date:** Oct 25, 2021
**To:** "Vignesh Narayanaswamy" [redacted]
**From:** "The Green Journal" em@greenjournal.org
**Subject:** Your Submission ONG-21-2084

RE: Manuscript Number ONG-21-2084

Breastfeeding infants receive neutralizing antibodies and cytokines via the milk of mothers immunized with a COVID-19 mRNA vaccine

Dear Dr. Narayanaswamy:

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

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

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

Your paper will be maintained in active status for 14 days from the date of this letter. If we have not heard from you by Nov 08, 2021, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1:

The authors' objective was to evaluate the immune response to COVID-19 mRNA-based vaccines present in breastmilk and the transfer of the immune response to the breastfeeding child. To accomplish this they conducted a prospective cohort study enrolled 30 lactating women who received an mRNA12 based COVID-19 vaccine between January and April 2021. Women provided serial milk samples, which included milk expressed before vaccination, milk expressed across 2-3 weeks after the first dose, and milk expressed across 3 weeks after the second dose. Women also provided their blood, spotted on cards (dried blood spots; DBS) 19 days after the first dose and 21 days after the second dose. Stool samples from the breastfed infants were collected 21 days after mothers received their second dose. Pre-pandemic samples of milk, DBS cards, and infant stool from prior studies were used as controls. Milk and infant stool samples were tested by ELISA for receptor-binding domain (RBD)-specific IgA and IgG. Milk samples were tested for the presence of neutralizing antibodies against the spike and four variants of concern (VOCs): D614G, B.1.1.7 (alpha), B.1.351 (beta), and P.1 (gamma). Levels of 10 cytokines were measured in milk samples. They found that Milk from COVID-19-immunized women neutralized the spike and four VOCs and this response is primarily IgG-driven. The immune response in milk also included significant elevation of interferon-γ (IFN-γ). The immune response to maternal vaccination was reflected in breastfed babies; anti-RBD IgG and anti-RBD IgA was detected in 33% and 30% of infant stool samples, respectively. Levels of anti-RBD antibodies in infant stool correlated with maternal vaccine side-effects.

I have a few questions and comments for the authors:
1. In the abstract, the control arm data seems absent?
2. How was the number of women to enroll decided on?
3. Why were women included in the study arm who had previously been infected with SARS-CoV-2?
4. Why did the authors include women in the study arm who had already been vaccinated?
5. How did the authors decide on the various timepoints for sample collection?
6. What kind of quality control was in place for the self-collected samples?
7. The degree of immunology jargon may at times be unfamiliar to the practicing obstetrician. Would the authors consider brief descriptions of such things as: "interaction of spike and its variant," or "receptor-binding domain-specific IgA and IgG," or the significance in this setting of the IgA response?
8. Was an attempt made to diversify the cohort studied (90% white)?
9. How did the authors select the thresholds for positivity for RBD-specific antibodies (set at OD values 3 times above standard deviation).

10. The authors report an extensive and varied set of immunologic responses covering approximately 5 pages of detailed results. In addition to 7 appendices and 5 figures. The net effect for the reader may be losing the forest for the trees, and difficulty in constructing a broader understanding of impact. I would suggest a restructuring of this section finding a better balance between narrative and figures and more clearly highlighting the most salient and dominant results.
11. Could the authors expound a little more on the variability noted in the neutralization ability of milk?
12. Can the authors expound more on why only a third of infant stool samples demonstrated detectable levels of anti-RBD IgA and IgG.
13. As in the abstract, the purpose and data from the control arm is unclear.

Reviewer #2:

This study examines immune response to COVID-19 mRNA in lactating people. Multiple responses are assessed (patient blood, breast milk, and infant stool). The methodology is well described and contributes novel data regarding antibody neutralization capacity and antibody presence in infant stool. My primary concerns are with the framing of the study, both in the introduction and the discussion. These concerns can easily be addressed with minimal revision.
1. Abstract, methods: you state you collect blood and stool but do not state what you do with them.
2. Introduction, lines 35-44: This paragraph significantly overstates the lack of data in a way that makes it seem risky to be vaccinated while breastfeeding. We've already learned that this sort of language in academic manuscripts will be coopted by people who are against vaccination. I understand your point, but can you acknowledge V-SAFE data that is reassuring and there is unlikely to be harm given the physiology involved?
3. Introduction, lines 42-44: Consider stating that at the time of this publication vaccination has not been approved for children under the age of 12. At some point, infants at 6 months or greater will likely be eligible.
4. Methods, lines 125-129: I'd like to see more detail regarding the protocol for detection of RBD-specific immunoglobulins. Also, I suspect these are human SARS-CoV-2 RBD ELISA kits. Explicitly state this.
5. Discussion: What do you make of the pre-pandemic milk samples testing positive for anti-RBD IgA and IgG? Please comment on this.
6. Discussion: Please acknowledge that the pearson's r for correlation of RBD-specific IgG and neutralizing ability is weak.
7. Discussion, lines 334-340: This final paragraph reads as though you are trying to convince people to avoid vaccination in pregnancy and wait until they are postpartum. Given the current state of morbidity and mortality amongst pregnant people secondary to COVID-19, I find this dangerous. I believe you can argue for the benefits of vaccination during breastfeeding without denigrating vaccination during pregnancy.

EDITORIAL OFFICE COMMENTS:

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

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

2. When you submit your revised manuscript, please make the following edits to ensure your submission contains the required information that was previously omitted for the initial double-blind peer review:
* Include your title page information in the main manuscript file. The title page should appear as the first page of the document. Add any previously omitted Acknowledgements (ie, meeting presentations, preprint DOIs, assistance from non-byline authors).
* Funding information (ie, grant numbers or industry support statements) should be disclosed on the title page and in the body text. For industry-sponsored studies, the Role of the Funding Source section should be included in the body text

of the manuscript.
* Include clinical trial registration numbers, PROSPERO registration numbers, or URLs at the end of the abstract (if applicable).
* Name the IRB or Ethics Committee institution in the Methods section (if applicable).
* Add any information about the specific location of the study (ie, city, state, or country), if necessary for context.

3. Obstetrics & Gynecology uses an "electronic Copyright Transfer Agreement" (eCTA), which must be completed by all authors. When you uploaded your manuscript, each co-author received an email with the subject, "Please verify your authorship for a submission to Obstetrics & Gynecology." Please check with your coauthors to confirm that they received and completed this form, and that the disclosures listed in their eCTA are included on the manuscript's title page.

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

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

6. Responsible reporting of research studies, which includes a complete, transparent, accurate and timely account of what was done and what was found during a research study, is an integral part of good research and publication practice and not an optional extra. Obstetrics & Gynecology supports initiatives aimed at improving the reporting of health research, and we ask authors to follow specific guidelines for reporting randomized controlled trials (ie, CONSORT), observational studies (ie, STROBE), observational studies using ICD-10 data (ie, RECORD), meta-analyses and systematic reviews of randomized controlled trials (ie, PRISMA), harms in systematic reviews (ie, PRISMA for harms), studies of diagnostic accuracy (ie, STARD), meta-analyses and systematic reviews of observational studies (ie, MOOSE), economic evaluations of health interventions (ie, CHEERS), quality improvement in health care studies (ie, SQUIRE 2.0), and studies reporting results of Internet e-surveys (CHERRIES). Include the appropriate checklist for your manuscript type upon submission. Please write or insert the page numbers where each item appears in the margin of the checklist. Further information and links to the checklists are available at http://ong.editorialmanager.com. In your cover letter, be sure to indicate that you have followed the CONSORT, MOOSE, PRISMA, PRISMA for harms, STARD, STROBE, RECORD, CHEERS, SQUIRE 2.0, or CHERRIES guidelines, as appropriate.

7. 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.

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

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).
* 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]."

10. The most common deficiency in revised manuscripts involves the abstract. Be sure there are no inconsistencies between the Abstract and the manuscript, and that the Abstract has a clear conclusion statement based on the results found in the paper. Make sure that the abstract does not contain information that does not appear in the body text. If you submit a revision, please check the abstract carefully.

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

11. 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.

12. 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.

13. In your Abstract, manuscript Results sections, and tables, the preferred citation should be in terms of an effect size, such as odds ratio or relative risk or the mean difference of a variable between two groups, expressed with appropriate confidence intervals. When such syntax is used, the P value has only secondary importance and often can be omitted or

noted as footnotes in a Table format. Putting the results in the form of an effect size makes the result of the statistical test more clinically relevant and gives better context than citing P values alone.

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

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

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

15. Please review examples of our current reference style at http://ong.editorialmanager.com (click on the Home button in the Menu bar and then "Reference Formatting Instructions" document under "Files and Resources). Include the digital object identifier (DOI) with any journal article references and an accessed date with website references. Unpublished data, in-press items, personal communications, letters to the editor, theses, package inserts, submissions, meeting presentations, and abstracts may be included in the text but not in the reference list.

In addition, the American College of Obstetricians and Gynecologists' (ACOG) documents are frequently updated. These documents may be withdrawn and replaced with newer, revised versions. If you cite ACOG documents in your manuscript, be sure the references you are citing are still current and available. Check the Clinical Guidance page at https://www.acog.org/clinical (click on "Clinical Guidance" at the top). If the reference is still available on the site and isn't listed as "Withdrawn," it's still a current document.

If the reference you are citing has been updated and replaced by a newer version, please ensure that the new version supports whatever statement you are making in your manuscript and then update your reference list accordingly (exceptions could include manuscripts that address items of historical interest). If the reference you are citing has been withdrawn with no clear replacement, please contact the editorial office for assistance (obgyn@greenjournal.org). In most cases, if an ACOG document has been withdrawn, it should not be referenced in your manuscript.

16. Figure 1: Please upload as a figure file on Editorial Manager.
Figure 2: Please break A and B into two different figures (Figure 2 A and B and Figures 3A-E). Please upload as figure files on Editorial Manager.
Figure 3: Please upload as a figure file on Editorial Manager.
Figure 4: Please upload as a figure file on Editorial Manager.
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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.

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

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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 as a Microsoft Word document. 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 Nov 08, 2021, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,
Dwight J. Rouse, MD
Associate Editor, Obstetrics

2020 IMPACT FACTOR: 7.661
2020 IMPACT FACTOR RANKING: 3rd out of 83 ob/gyn journals

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UNIVERSITY OF MASSACHUSETTS
AMHERST
Life Sciences Laboratory 1 Room N529
240 Thatcher Road
Amherst, MA 01003-9298

[redacted]

November 8, 2021

Dear Editor(s)

We thank the reviewers for their generous comments on our manuscript and have edited the manuscript to address their concerns. In our responses, we have included line numbers as necessary, and all corresponding changes in the manuscript text are tracked.

The manuscript is not under consideration for publication in any other journal and will not be submitted until a final negative decision is received from *Obstetrics & Gynecology*. The study was IRB-approved at UMass Amherst and individuals provided consent prior to participation. Permission was obtained from Rachel Taylor, Leah Driscoll, and Riley Burke, named in the acknowledgements. The manuscript was posted online on medRxiv (https://doi.org/10.1101/2021.10.12.21264890)

Transparency declaration statement: *I, Kathleen Arcaro, 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.*

Statement attesting to self-blinding: *I, Kathleen Arcaro, have reviewed and edited the submission to omit any identifying information. I hereby submit this self-blinded manuscript for consideration in Obstetrics & Gynecology.*

We believe that the manuscript is now suitable for publication in *Obstetrics & Gynecology*.

Sincerely,

Kathleen F. Arcaro

Kathleen F. Arcaro, Professor

**REVIEWER COMMENTS:**

**Reviewer #1:**

The authors' objective was to evaluate the immune response to COVID-19 mRNA-based vaccines present in breastmilk and the transfer of the immune response to the breastfeeding child. To accomplish this they conducted a prospective cohort study enrolled 30 lactating women who received an mRNA12 based COVID-19 vaccine between January and April 2021. Women provided serial milk samples, which included milk expressed before vaccination, milk expressed across 2-3 weeks after the first dose, and milk expressed across 3 weeks after the second dose. Women also provided their blood, spotted on cards (dried blood spots; DBS) 19 days after the first dose and 21 days after the second dose. Stool samples from the breastfed infants were collected 21 days after mothers received their second dose. Pre-pandemic samples of milk, DBS cards, and infant stool from prior studies were used as controls. Milk and infant stool samples were tested by ELISA for receptor-binding domain (RBD)-specific IgA
and IgG. Milk samples were tested for the presence of neutralizing antibodies against the spike and four variants of concern (VOCs): D614G, B.1.1.7 (alpha), B.1.351 (beta), and P.1 (gamma). Levels of 10 cytokines were measured in milk samples. They found that Milk from COVID-19-immunized women neutralized the spike and four VOCs and this response is primarily IgG-driven. The immune response in milk also included significant elevation of interferon-γ (IFN-γ). The immune response to maternal vaccination was reflected in breastfed babies; anti-RBD IgG and anti-RBD IgA was detected in 33% and 30% of infant stool samples, respectively. Levels of anti-RBD antibodies in infant stool correlated with maternal vaccine side-effects.

Please see below our responses to the reviewers' comments

1. In the abstract, the control arm data seems absent

**We thank the reviewer for pointing this out. We have included the control arm data set in the abstract (lines 64-65).**

2. How was the number of women to enroll decided on?

**Enrollment was open with the goal of recruiting a minimum of 10 women for each vaccine brand. The manuscript text has been modified accordingly in the methods section (lines 103-104).**

3. Why were women included in the study arm who had previously been infected with SARS-CoV-2?

**COVID-19 vaccination is being recommended for breastfeeding women regardless of whether they had COVID, so we considered it appropriate to retain infected/recovered women in our study.**

4. Why did the authors include women in the study arm who had already been vaccinated?

**All women enrolled in the study had to be vaccinated in order to participate. Our outreach did not specify that women had to enroll before getting a COVID-19 mRNA vaccine. We enrolled women who either received a vaccine dose or were scheduled to receive one. In the**

**case where women already received their first vaccine dose, we requested a milk sample saved by women before they got their first dose.**

5. How did the authors decide on the various timepoints for sample collection?

**We decided on sample collection timepoints based on the following:**

- **The vaccination schedule set by Moderna and Pfizer**
- **To capture any early changes encompassing cytokines and vaccine-related side effects**
- **To capture any mature antibody response after women received their second vaccine dose**
- **To limit the burden on the participating women**

**This resulted in a fairly comprehensive sample set with samples collected at ~ 3 day intervals (see Appendix 1), the full set was analyzed for RBD antibodies by ELISA but only a subset of samples were analyzed for neutralization capacity and cytokines as specified in the text.**

6. What kind of quality control was in place for the self-collected samples?

**Identical kits were provided to all participants, comprising 30 mL containers that were dated for sample collection, two icepacks to be put in participant's freezer at least 24 hours before shipment to UMass, and detailed instructions on milk, stool, and dried blood spot collection. Additionally, women completed surveys on REDCap (Research Electronic Data Capture) recording their dates of sample collection, maternal and infant side effects to the vaccine, and general health-related information.**

7. The degree of immunology jargon may at times be unfamiliar to the practicing obstetrician. Would the authors consider brief descriptions of such things as: "interaction of spike and its variant," or "receptor-binding domain-specific IgA and IgG," or the significance in this setting of the IgA response?

**We have added some explanatory comments in the text (lines 162-164, 274-276)**

8. Was an attempt made to diversify the cohort studied (90% white)?

**To facilitate recruitment of women of all races, the study was posted on our website (www.breastmilkresearch.org) and we performed additional outreach to staff at the affiliated hospital. We have included statements to this effect in lines 216-218.**

9. How did the authors select the thresholds for positivity for RBD-specific antibodies (set at OD values 3 times above standard deviation).

**The positive cut-off value was set based on methodology from published literature[1,2]. Appropriate references are cited in line 198 (references 20 and 21)**

10. The authors report an extensive and varied set of immunologic responses covering approximately 5 pages of detailed results. In addition to 7 appendices and 5 figures. The net effect for the reader may be losing the forest for the trees, and difficulty in constructing a broader understanding of impact. I would suggest a restructuring of this section finding a better balance between narrative and figures and more clearly highlighting the most salient and dominant results.

**Thank you for this suggestion. We have restructured the manuscript accordingly.**

- **We moved the Spike neutralization (old Fig. 3) to Appendix 5 which also has the neutralization data for variants (note Reviewer 2 had comments about the limited correlation).**
- **We refocused the cytokine section by limiting comments about cytokines other than IFNγ (lines 297-311).**
- **We trimmed and reorganized the section on antibody in infant stool, in particular we moved and reworked comments about total immunoglobulins (lines 287-293).**

11. Could the authors expound a little more on the variability noted in the neutralization ability of milk?

**The third paragraph of the discussion addresses the variability of milk samples to neutralize the spike panel. Note that we delve into the patterns of response in Appendix 8 that further helps explain this variability.**

12. Can the authors expound more on why only a third of infant stool samples demonstrated detectable levels of anti-RBD IgA and IgG.

**We think that disgestion within the baby gut is a likely factor, especially for IgG which lacks the protections of secretory IgA forms. Additionally, low levels of anti-RBD antibodies in a subset of infant stool samples can be attributed to transcytosis of anti-RBD antibodies from the infant instestine to circulation[3,4]. We have modified the discussion accordingly (lines 368-371)**

13. As in the abstract, the purpose and data from the control arm is unclear.

**We have clarified the text to indicate that pre-pandemic samples establish a baseline (line 109-110).**

**Reviewer #2:**

This study examines immune response to COVID-19 mRNA in lactating people. Multiple responses are assessed (patient blood, breast milk, and infant stool). The methodology is well described and contributes novel data regarding antibody neutralization capacity and antibody presence in infant stool. My primary concerns are with the framing of the study, both in the introduction and the discussion. These concerns can easily be addressed with minimal revision.

1. Abstract, methods: you state you collect blood and stool but do not state what you do with them.
**We thank the reviewer for this comment. We have edited the abstract accordingly (lines 54-55)**

2. Introduction, lines 35-44: This paragraph significantly overstates the lack of data in a way that makes it seem risky to be vaccinated while breastfeeding. We've already learned that this sort of language in academic manuscripts will be coopted by people who are against vaccination. I understand your point, but can you acknowledge V-SAFE data that is reassuring and there is unlikely to be harm given the physiology involved?
**We thank the reviewer for this comment. We agree that the paragraph in its original form seemed as though COVID-19 vaccines pose a risk while breastfeeding. We have modified this section accordingly.**

3. Introduction, lines 42-44: Consider stating that at the time of this publication vaccination has not been approved for children under the age of 12. At some point, infants at 6 months or greater will likely be eligible.
**We have added the necessary information in lines 75-77**

4. Methods, lines 125-129: I'd like to see more detail regarding the protocol for detection of RBD-specific immunoglobulins. Also, I suspect these are human SARS-CoV-2 RBD ELISA kits. Explicitly state this.
**We cite our previously published paper (reference 19) for a detailed protocol for detection of anti-RBD immunoglobulins. This was an assay developed and validated in-house and we have included a statement to that effect in the methods section (line 162)**

5. Discussion: What do you make of the pre-pandemic milk samples testing positive for anti-RBD IgA and IgG? Please comment on this.
**We thank the reviewer for this comment. We agree that it is important to address pre-pandemic samples that scored positive for anti-RBD antibodies. Among the pre-pandemic milk samples testing positive for anti-RBD IgA and IgG, a prior infection in women who provided these samples could have elicited a humoral (antibody) response that cross-reacted with SARS-CoV-2-RBD. We have modified the discussion accordingly, with appropriate references (lines 333-337).**

6. Discussion: Please acknowledge that the pearson's r for correlation of RBD-specific IgG and neutralizing ability is weak.

**We have acknowledged the weak Pearson's R in the results section (line 279), the old Fig 3 with the neutralization data for Spike vs RBD IgG has been moved to Appendix 5 (added to the panels for variants). This de-emphasises the topic and also simplified the results section as requested by Reviewer 1.**

7. Discussion, lines 334-340: This final paragraph reads as though you are trying to convince people to avoid vaccination in pregnancy and wait until they are postpartum. Given the current state of morbidity and mortality amongst pregnant people secondary to COVID-19, I find this dangerous. I believe you can argue for the benefits of vaccination during breastfeeding without denigrating vaccination during pregnancy.

**We thank the reviewer for this excellent point. Our intentions were not to denigrate vaccination during pregnancy. We have modified the text accordingly (lines 378-381).**

## References


1. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol*. 2020;57(1):1-15. doi:10.1002/cpmc.100
2. Fox A, Marino J, Amanat F, et al. Robust and specific secretory IgA against SARS-CoV-2 detected in human milk. *iScience*. 2020;23(11):101735. doi:10.1016/j.isci.2020.101735
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