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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: 10/21/2022
To: "Brett David Einerson" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-22-1625

RE: Manuscript Number ONG-22-1625

Ultrasound of the explanted uterus in placenta accreta spectrum: insights into pathophysiology

Dear Dr. Einerson:

Thank you for sending us your work for consideration for publication in Obstetrics & Gynecology. Your manuscript has been reviewed by the Editorial Board and by special expert referees. The Editors would like to invite you to submit a revised version for further consideration.

If you wish to revise your manuscript, please read the following comments submitted by the reviewers and Editors. Each point raised requires a response, by either revising your manuscript or making a clear argument as to why no revision is needed in the cover letter.

To facilitate our review, we prefer that the cover letter you submit with your revised manuscript include each reviewer and Editor comment below, followed by your response. That is, a point-by-point response is required to each of the EDITOR COMMENTS (if applicable), REVIEWER COMMENTS, and STATISTICAL EDITOR COMMENTS (if applicable) below.

The revised manuscript should indicate the position of all changes made. Please use the "track changes" feature in your document (do not use strikethrough or underline formatting).

Your submission will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by 11/11/2022, we will assume you wish to withdraw the manuscript from further consideration.

EDITOR COMMENTS:

Please note the following:

* Help us reduce the number of queries we add to your manuscript after it is revised by reading the Revision Checklist at https://journals.lww.com/greenjournal/Documents/RevisionChecklist_Authors.pdf and making the applicable edits to your manuscript.

* Figures 1-6: Please upload versions without A, B, C, etc. labels to Editorial Manager. These will be added back per journal style.

REVIEWER COMMENTS:

Reviewer #1: The investigators studied intraoperative photography, sonography of explanted uteruses, and surgical pathology among 34 women who underwent hysterectomy for placenta accreta spectrum (PAS) in order to "better understand placenta accreta spectrum". The study is written and presented well, and novel. Observation 4, essentially that pathologic findings that suggest the serosa was breached were actually the result of surgical manipulation, is particularly novel and useful.

Comments and suggestions:

1. Of greatest concern, no or limited data are given regarding how frequently specific findings were or were not seen to support each of the 4 formal observations. As a study in a research journal, empiricism remains important even if the study is described as "observational." How were intraoperative observations made and recorded? How often was each identified or not identified among each of the described 4 observations?

2. The study methodology is somewhat unclear. Earlier, methodology is described as a correlation between the three

modalities, but later the state is made that "When possible, correlation was made between the intraoperative photos, the ultrasound images of the explanted uterus, and gross pathologic evaluation."

3. Given the centrality of sonography of uterine explants to this study, please include greater detail about how this is preformed than simply that the specimen "was placed in a water bath". Was the water bath sealed? How could one replicate this technique?

4. This paper would benefit from a sentence in the Introduction about why sonography of uterine explants was pursued and is advantageous (demonstrated by the image clarity shown in figure 1c); presently, the advantage is mentioned only in the Discussion.

5. Only those with PAS grade 2 and 3 were included. Observation 2 is that all cases showed some degree of placental bulging; was this not predisposed by study design?

6. Some aspects of the conclusion, such as a call for preoperative imaging to identify pelvic adhesive disease, and why, do not match what the study addressed.
a. Sonography or MRI are unlikely to identify pelvic adhesive disease. This also suggests the study was aimed at better prediction of PAS, whereas the presentation really focuses on an understanding of mechanism of PAS.
b. The call to abandon diagnostic focus on depth of invasion is not supported by these data, which do not at all mention maternal outcomes such as hemorrhage or other major morbidities.

Minor:

While intriguing, the title does not entirely reflect the conduct or purpose of this study, highlighting only sonography of the explanted uterus rather than additional correlation with intraoperative photography and pathology with purpose of understanding PAS.

Reviewer #2: This is a very clearly written manuscript. The study design is novel, clearly very systematic and the conclusions are supported by the data.

I have the following recommendations/comments:

1) Line 42- The authors report the risk of maternal mortality as high as 7%, especially when unanticipated. While this is true, this statistic is from a 1996 paper (the newer one cited in this manuscript is not the original source of this statistic, please revise: O'Brien JM, Barton JR, Donaldson ES. The management of placenta percreta: conservative and operative strategies. Am J Obstet Gynecol. 1996;175(6):1632-1638. doi:10.1016/s0002-9378(96)70117-5). Please include this original source, so that readers and future authors are clear about this statistic. I am unaware of any more recent reports of maternal mortality other than the following:

A recent meta-analysis the rate of maternal mortality is closer to 1.3% (Jauniaux E, Grønbeck L, Bunce C, Langhoff-Roos J, Collins SL. Epidemiology of placenta previa accreta: a systematic review and meta-analysis. BMJ Open. 2019;9(11):e031193. Published 2019 Nov 12. doi:10.1136/bmjopen-2019-031193). Please include this range to give a more accurate description for future reference by other authors.

2) Lines 120-121- Some may question the feasibility of retrospectively reassigning cases completed prior to adoption of FIGO clinical classifcation. Consider including a reference to this manuscript, which showed the correlation between retrospective assignment of FIGO Classification and histopathologic findings: Aalipour S, Salmanian B, Fox KA, et al. Placenta Accreta Spectrum: Correlation between FIGO Clinical Classification and Histopathologic Findings [published online ahead of print, 2021 May 2]. Am J Perinatol. 2021;10.1055/s-0041-1728834. doi:10.1055/s-0041-1728834.

3) In Table 2, please include the breakdown of the histopathology classifcation 3A, 3D, 3E, as it is referenced in the legend of the table (lines 361-362). I also recommend showing the breakdown of FIGO classification and Histopath side by side for easier visual comparison.

4) The strengths of the study are well described. There are additional limitations, that I recommend addressing. Specifically, the authors cite (and show in the perioperative imaging) that vascular remodeling contributes to morbidity. It is bearkt impossible to see this vascularity well in the uterine explant, after vascular flow has been disrupted. This will not be apparent on post-explant ultrasound. Comparison of pre- and post-surgical imaging would strengthen this point, and highlight the limitations of using ONLY histopathologic diagnosis (which the authors nicely state is not useful for surgical management, as this occurs after management is complete). Perhaps this comparison Pre/post imaging is worthy of a separate manuscript, but very important.

5) The authors report getting photos of the uterus and explant "from all angles." Please include instructions of precisely how these photos were taken (perhaps in a supplement) to allow others to replicate/validate.

Reviewer #3: The authors set out to prospectively characterize placenta accrete spectrum (PAS) through visual inspection, sonography, and pathological assessment.

The major limitation of this analysis is the exclusion of Grade 1 PAS. To better inform the hypothesis that PAS is a problem of uterine dehiscence as opposed to invasion having this "early stage" of PAS included could shed light on progression or other pathophysiology.

Introduction: Concise description of the problem. Gaps well highlighted. Aim/objective clearly stated.

Methods:Why not include Grade 1 PAS?

Results: Observations tend to include more than 1 observance. For example observation #1 i) uteroplacental interface smooth and distinct 2) myometrium imperceptible 3) placenta confined by smooth "scar shell." Perhaps a subheading for each may be helpful (e.g. scar shell)

Regarding observation #2 "every specimen showed placental bulging through the lower uterine segment in the region of prior hysterectomy." The authors controlled for location of hysterotomy so I'm not sure how valid this finding is.

Additionally, observation #3 contends there was no true bladder invasion. Could this also be due to limitation based on inclusion criteria. For example emergency cases may have shown something different.

Discussion: Authors should attempt to speculate and describe what is happening in grade 1 PAS based on these observations

Tables: The table is wordy. Consider bullets or simplifying data.

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Sincerely,
Jason D. Wright, MD
Editor-in-Chief

The Editors of Obstetrics & Gynecology

To the Editors and Reviewers at *Obstetrics & Gynecology*,

Thank you for the opportunity to revise and resubmit our manuscript previously entitled *Ultrasound of the explanted uterus in placenta accreta spectrum: insights into pathophysiology*. We have addressed each of the editor and reviewer comments (**below**), revised the manuscript, and now find the manuscript to be much improved to the original submission. We hope that you will agree.

Sincerely,

Brett D. Einerson, MD MPH
Corresponding author

EDITOR COMMENTS:

Please note the following:

*** Help us reduce the number of queries we add to your manuscript after it is revised by reading the Revision Checklist at https://journals.lww.com/greenjournal/Documents/RevisionChecklist_Authors.pdf and making the applicable edits to your manuscript.**

*** Figures 1-6: Please upload versions without A, B, C, etc. labels to Editorial Manager. These will be added back per journal style.**
This has been done.

REVIEWER COMMENTS:

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We appreciate these positive comments.

Comments and suggestions:

1. Of greatest concern, no or limited data are given regarding how frequently specific findings were or were not seen to support each of the 4 formal observations. As a study in a research journal, empiricism remains important even if the study is described as "observational." How were intraoperative observations made and recorded? How often was each identified or not identified among each of the described 4 observations?
Thank you for this important comment regarding our methods. We modified our paper for clarity. The 4 observations were derived after interdisciplinary review of cases, and not determined *a priori*. They were, by our definition of what constitutes an observation, always identified (as opposed to not identified) in all cases. We provided a clearer definition of what constituted an 'observation' in line 139 and following in the methods: "Four main observations, constituting our results, emerged through comprehensive interdisciplinary case review by the team of radiologists, obstetricians and surgeons, and the pathologists constituting the authors. At the end of the study, we reviewed intraoperative photography, postoperative ultrasound, and gross pathology

for each individual case to be sure that the findings from that the findings were observed in all cases." We also provided clearer frequencies in the Table to address the reviewer concern, although this could be deleted for clarity and simplicity, since it is somewhat redundant with the text, at the editors' discretion.

2. The study methodology is somewhat unclear. Earlier, methodology is described as a correlation between the three modalities, but later the state is made that "When possible, correlation was made between the intraoperative photos, the ultrasound images of the explanted uterus, and gross pathologic evaluation."
We hope that the above revision in line 139 and following clarifies the methods in response to this question.

3. Given the centrality of sonography of uterine explants to this study, please include greater detail about how this is preformed than simply that the specimen "was placed in a water bath". Was the water bath sealed? How could one replicate this technique?
We revised the paragraph in the methods about how this technique was performed to increase the level of detail and clarity. This is found in line 108-126

4. This paper would benefit from a sentence in the Introduction about why sonography of uterine explants was pursued and is advantageous (demonstrated by the image clarity shown in figure 1c); presently, the advantage is mentioned only in the Discussion.
This is an excellent point. We added the following to line 66-68, "Sonography of the explanted uterus and placenta, we presumed, would provide a level of image resolution not possible *in situ* that could inform understanding of how PAS develops and exists in the body."

5. Only those with PAS grade 2 and 3 were included. Observation 2 is that all cases showed some degree of placental bulging; was this not predisposed by study design?
Yes. Essentially by definition PAS grade 1 is microscopically attached by not extending through the scar. This is clarified in line 170 and in the Table.

6. Some aspects of the conclusion, such as a call for preoperative imaging to identify pelvic adhesive disease, and why, do not match what the study addressed.
See in-line responses below. We hope that we have moderated and clarified the conclusions sufficiently. We are happy to perform further revisions at the reviewers and editors' request.

a. Sonography or MRI are unlikely to identify pelvic adhesive disease. This also suggests the study was aimed at better prediction of PAS, whereas the presentation really focuses on an understanding of mechanism of PAS.
We agree that our study did not address the association between individual observations (or findings like adhesive disease) and morbidity. It was too small to do so. But adhesive disease emerged as an important finding in our study for both Observation 3 and Observation 4 in describing these severe forms of PAS (which are more morbid than milder disease). To guide future inquiry, we speculate based on the findings of the study and our collective experience that adhesive disease plays a major role in mediating morbidity and propose this as a suitable alternative to findings previously emphasized that do not contribute to morbidity (e.g. searching for exophytic masses or quantifying the % depth of 'invasion'). We disagree that sonography is unlikely to identify pelvic adhesive disease, as several investigators are pursuing promising ways of accomplishing this. (PMID 29575202, 29420398). Of course, these are currently research protocols and not ready for routine clinical use.

If the editors agree, we propose to leave adhesive disease in the conclusion. We added those two references to reinforce the feasibility of identifying pelvic adhesive disease. We also added a moderating preamble to the paragraph in question, now reading in line 294 "Based on the findings presented here and our collective experience, we believe that imaging diagnosis of PAS should focus more on…"

b. The call to abandon diagnostic focus on depth of invasion is not supported by these data, which do not at all mention maternal outcomes such as hemorrhage or other major morbidities.
We agree that our study did not aim to correlate depth of invasion with hemorrhage and morbidity and we do not want to overstate our results. However, based on the findings (particularly Observation 1 and 2) we believe

that villous invasion or placental invasion is an invalid way of describing the pathophysiology of PAS. We state " relying on descriptions of placental invasiveness for grading and staging is problematic since, in our opinion, this is not actually how the disease progresses." This statement, we think, follows directly from the observations.

To address this important critique, we have added the following to the limitations (line 262-265): "this study was not large enough or intended to evaluate the association between individual findings and morbidity outcomes. It remains unclear which of the findings or observations is most directly related to hemorrhage and morbidity."

We also altered the description in question from "depth of invasion" to "characterizing placental invasion" (line 298).

Minor:

While intriguing, the title does not entirely reflect the conduct or purpose of this study, highlighting only sonography of the explanted uterus rather than additional correlation with intraoperative photography and pathology with purpose of understanding PAS.
We have revised the title to clarify that the findings related to understanding PAS were made using multiple modes of assessment, while still highlighting the most novel aspect of the approach (use of postoperative ultrasound). The revised title is: "Ultrasound of the explanted uterus in placenta accreta spectrum: correlation with intraoperative findings and gross pathology offers insights into pathophysiology."

Reviewer #2: This is a very clearly written manuscript. The study design is novel, clearly very systematic and the conclusions are supported by the data.
Thank you. We appreciate these comments.

I have the following recommendations/comments:

1) Line 42- The authors report the risk of maternal mortality as high as 7%, especially when unanticipated. While this is true, this statistic is from a 1996 paper (the newer one cited in this manuscript is not the original source of this statistic, please revise: O'Brien JM, Barton JR, Donaldson ES. The management of placenta percreta: conservative and operative strategies. Am J Obstet Gynecol. 1996;175(6):1632-1638. doi:10.1016/s0002-9378(96)70117-5). Please include this original source, so that readers and future authors are clear about this statistic. I am unaware of any more recent reports of maternal mortality other than the following:

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This statement is about *severe cases* specifically, and not all cases of previa-accreta. Nonetheless, we appreciate the comment and so have included a range since this is a better way of understanding the importance of the issue.

We didn't cite O'Brien originally because this statistic is now likely outdated (year 1996). It is now included.

We kept the Zuckerwise statistic because it is true to the meaning of the sentence. In *severe cases*, Zuckerwise showed 5-7% mortality rate in the delayed hysterectomy and immediate hysterectomy groups of this very modern cohort.

The 1.3% quoted by the reviewer is not exactly right, and likely underestimates the true mortality of *severe cases* since in this metaanalysis 60% were mild.

The true statistic is unknown, but likely greater than 1%.

The new sentence (underlined revision, line 40) reads: "Maternal morbidity is common, and death rates of 1.3% to 7% have been reported in severe cases."

2) Lines 120-121- Some may question the feasibility of retrospectively reassigning cases completed prior to adoption of FIGO clinical classifcation. Consider including a reference to this manuscript, which showed the correlation between retrospective assignment of FIGO Classification and histopathologic findings: Aalipour S, Salmanian B, Fox KA, et al. Placenta Accreta Spectrum: Correlation between FIGO Clinical Classification and Histopathologic Findings [published online ahead of print, 2021 May 2]. Am J Perinatol. 2021;10.1055/s-0041-1728834. doi:10.1055/s-0041-1728834.
Thank you for this comment. We clarified the text at line 130 as follows and added this citation.

"Cases performed prior to that publication were reclassified by FIGO grading based on operative reports descriptions and intraoperative photography (when photography was available).[24]"

3) In Table 2, please include the breakdown of the histopathology classifcation 3A, 3D, 3E, as it is referenced in the legend of the table (lines 361-362). I also recommend showing the breakdown of FIGO classification and Histopath side by side for easier visual comparison.
For simplicity we did not report subtypes of PAS Grade 3 in the original table, but now have done so (see Supplementary Table and details in the Table Legend). We did not show "breakdown of FIGO and Histopath side-by-side," as requested, since this would require creating a table with lines for each of the 34 cases. In our opinion, this would not add important information, but would result in considerable clutter. If the editors have another way to address this request, please do let us know.

4) The strengths of the study are well described. There are additional limitations, that I recommend addressing. Specifically, the authors cite (and show in the perioperative imaging) that vascular remodeling contributes to morbidity. It is bearkt impossible to see this vascularity well in the uterine explant, after vascular flow has been disrupted. This will not be apparent on post-explant ultrasound. Thank you for this important comment, which I believe states that it is impossible to see vascularity well in the explanted uterus. We have added this as a limitation in line 265-267: "although we documented hypervascularity intraoperatively for many cases, we were unable to assess blood flow in explanted specimens." **Comparison of pre- and post-surgical imaging would strengthen this point, and highlight the limitations of using ONLY histopathologic diagnosis (which the authors nicely state is not useful for surgical management, as this occurs after management is complete). Perhaps this comparison Pre/post imaging is worthy of a separate manuscript, but very important.** We are thankful to the reviewer for this reinforcement. We agree that this topic is worthy of a separate manuscript to tie postoperative findings to preoperative findings. No changes were made to the manuscript in response to this last comment.

5) The authors report getting photos of the uterus and explant "from all angles." Please include instructions of precisely how these photos were taken (perhaps in a supplement) to allow others to replicate/validate.
We prepared a supplement demonstrating standard views of the explanted uterus in a representative case (see Supplementary Figure).

Reviewer #3: The authors set out to prospectively characterize placenta accrete spectrum (PAS) through visual inspection, sonography, and pathological assessment.

The major limitation of this analysis is the exclusion of Grade 1 PAS. To better inform the hypothesis that PAS is a problem of uterine dehiscence as opposed to invasion having this "early stage" of PAS included could shed light on progression or other pathophysiology.
We appreciate this comment. We clarified that our purpose was to understand the pathophysiology of "more severe types of PAS" It is, no doubt, a subtle distinction, but we presumed that PAS Grade 1 would not have striking postoperative ultrasound characteristics since it is, by Hecht definition, microscopic (not visible on ultrasound or gross pathology) and "noninvasive." As such, our methods of visual intraoperative inspection, ultrasound, and gross pathologic imaging, were focused on more advanced disease "based on the degree of grossly assessed invasion" (Hecht et al). We added this point to the limitations in line 260 and following, "Since our assessments focused on gross and sonographic findings of severe PAS, we also cannot comment specifically on the pathophysiology of mild or "noninvasive" subtypes (PAS Grade 1) as these are, by definition, microscopic."

Introduction: Concise description of the problem. Gaps well highlighted. Aim/objective clearly stated.
Thank you for this comment.

Methods: Why not include Grade 1 PAS?
Please see our response to the above.

Results: Observations tend to include more than 1 observance. For example observation #1 i) uteroplacental interface smooth and distinct 2) myometrium imperceptible 3) placenta confined by smooth "scar shell." Perhaps a subheading for each may be helpful (e.g. scar shell)
This format change is reasonable, and we could do this if the editors agree. In our opinion, a narrative description of the findings is more effective, as written. No changes were made to the manuscript in response.

Regarding observation #2 "every specimen showed placental bulging through the lower uterine segment in the region of prior hysterectomy." The authors controlled for location of hysterotomy so I'm not sure how valid this finding is.
We believe this observation is valid based on the findings. Making this distinction is important, since PAS is still more-commonly-than-not described in the literature and textbooks as progressing by villous invasion, and often uterine scar dehiscence is mentioned as a rare subtype or separate process altogether. We interpret that this finding (observation 2) challenges that assumption (as stated in the text and table) and aim to redefine uterine scar dehiscence as a "central feature of PAS development… in lower uterine segment PAS." No changes were made to the manuscript in response.

Additionally, observation #3 contends there was no true bladder invasion. Could this also be due to limitation based on inclusion criteria. For example emergency cases may have shown something different.
We should clarify that emergency cases were not excluded; only those that were not diagnosed antenatally. Thus, this limitation is not applicable. In response, we deleted "~~resulting in emergency surgery~~" from the description of cases of unsuspected or missed diagnoses in line 163.

Discussion: Authors should attempt to speculate and describe what is happening in grade 1 PAS based on these observations
We did not speculate in the original draft, as our findings were focused on gross (not microscopic) descriptions of PAS Grade 2 and 3. We added the following to the limitations in line 260-261 "cannot comment specifically on the pathophysiology of mild or 'noninvasive subtypes (PAS Grade 1) as these are, by definition, microscopic."

Tables: The table is wordy. Consider bullets or simplifying data.
We would be happy to do this if the editors agree. In this revision, we have not done so, since we believe the full description, as well as interpretations, are necessary and helpful in table format. No changes were made to the manuscript in response.