OBSTETRICS & GYNECOLOGY

NOTICE: This document contains comments from the reviewers and editors generated during peer review of the initial manuscript submission and sent to the author via email.

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

Date: Jan 31, 2020
To: "Amy L. Harris" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-20-70

RE: Manuscript Number ONG-20-70

Vanishing Twins Conceived Through In Vitro Fertilization: Obstetrical Outcomes and Placental Pathology

Dear Dr. Harris:

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 Feb 21, 2020, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1: Harris and colleagues present a retrospective cohort study evaluating obstetric outcomes and placental pathology between IVF pregnancies with vanishing twin syndrome and IVF twins/singletons. I have the following questions/comments for the authors.

1 - The manuscript is well written and clear.

2 - The topic is interesting and as many patients with IVF go on to be cared for by general OBGYNs, the appropriateness for this journal is increased. However, the detail to which the placental pathology is explored may be less appropriate as the possible clinical significance of the findings was not demonstrated in the obstetric outcomes. Consider an abbreviated discussion regarding the placental findings.

3 - The strict definition of VTS is noted and rational explained. However, as the alternative of an empty second sac does arise in both IVF and spontaneous pregnancies, it may have been interesting to include this as a subgroup if there were adequate numbers.

4 - The authors included ICSI cycles. The exclusion of frozen transfer was explained with evidence of difference in outcomes between fresh and frozen. Has any such association been shown with ICSI since there is alteration to the oocytes that occurs? Seems as though if this is an unknown, it could possibly be confounding results.

5 - Bold significant p values in Table 1 and 3 as these are a bit busy and that will help with ease of reading.

Reviewer #2: "The aim of this study was to evaluate differences in obstetric outcomes and placental pathology between vanishing twin pregnancies and singleton or twin pregnancies in a large cohort of IVF pregnancies."

117: This was a retrospective study from 2004-2016. I wonder about changes in IVF protocol over the 12 year period and whether any changes might affect outcomes to be measured, such as assisted hatching, PGS, PGD, etc.

136: "The decision of number of embryos to transfer were guided by clinical prognostic factors including: female partner age, prior infertility treatment history, embryo quality, and availability of excess embryos available for cryopreservation and followed institutional and national guidelines (26-29)."

We know that placental pathology is different when comparing single embryo transfer resulting in twin gestation and more than two embryo transfer. Therefore I believe these may be important factors that might confound the comparison of perinatal outcomes and placental pathology comparisons. The title should reflect that all embryos transferred were fresh IVF embryos.

142: "For the purposes of this analysis, vanishing twin syndrome was defined as a first trimester ultrasound demonstrating two gestational sacs, each with a yolk sac, followed by an ultrasound illustrating only one fetal heartbeat. Patients with an empty second gestational sac at their first ultrasound were not considered a vanishing twin pregnancy."

As you are aware, the definition of "vanishing twin pregnancy" has multiple differing interpretations (Landy and Keith, 1998; Pinborg et al., 2006, Pinborg, 2007). Various definitions include those from a 1st trimester loss of an empty gestational sac of one of the two pregnancies (La Sala et al., 2006) to the demise of a twin fetus occurring at later gestational ages. As a result of the differing definitions of "vanishing twin pregnancy making a comparison of perinatal outcome and placental pathology would be difficult to interpret and probably represent an invalid comparison with other studies of vanishing twin pregnancies.

258: "Significantly greater anatomic pathology in VTS placentas compared to singleton and twin placentas persisted when confounders were controlled in our analyses."

To make an accurate comparison of anatomic pathology in VTS, I would suggest comparing outcomes and pathology based on the number of embryos transferred, the embryo stage of transfer (blastocyst versus cleavage stage embryo), the timing of transfer. and whether the embryos followed spontaneous insemination or ICSI. We know that perinatal outcomes following single embryo transfer resulting in an early twin pregnancy are different when compared with multiple embryo transfers as well pregnancies following spontaneous insemination compared with ICSI (Royster, 2016) and would certainly confound the interpretation regarding perinatal outcomes and differences in placental pathology.

An additional confounding variable that was not clearly discussed was the chorionicity of vanishing twin pregnancies versus ongoing twin pregnancies. Were the single embryo transfer pregnancies resulting in twin pregnancy monochorionic or dichorionic? An additional confounding variable to consider when looking at outcomes and placental pathology.

326: Mean ages of 34-36, I presume a significant number of patients underwent aneuploidy testing such as PGS, PGD, CVS or Amnio. Any thought into comparing outcomes based on presence or absence aneuploidy testing?

In conclusion: I think your study is interesting but from the data and information presented I believe there are too many confounders involved in your patients to make the following conclusion: : "IVF pregnancies affected by VTS did not have significant differences in obstetric or perinatal outcomes as compared to twin or singleton gestations. However, early twin loss potentially impacts placental development leading to a higher rate of small placentas and other anatomic pathologies."

Confounders being: non-standard definition of "vanishing twin pregnancy". number of embryos transferred, embryonic stage of embryo transferred, timing of transfer on day 2-5, spontaneous insemination versus ICSI, preimplantation testing with PGS or PGD, and chorionicity of twin pregnancies.

Reviewer #3:

1. This is a well-written retrospective study of IVF pregnancies that compares both obstetric outcomes and placental pathology characteristics between singleton, twin and pregnancies affected by a vanishing twin. The careful attention to how vanishing twin is specifically defined and the comparable population (as they are all fresh IVF cycle pregnancies) is great.

2. One issue is that the distinction between monochorionic and dichorionic twin pregnancies is not made. This distinction could make a big difference in the VTS group (due to placental anastomoses) and of course to the ongoing twin groups. One option is to exclude monochorionicity or to separate by chorionicity or to include it as a subgroup (similar to the subgroup comparing >8w VTS.

3. In addition to comparing infant birth weight, could you add a comparison of the birth weight percentiles corrected for gestational age of birth because looking at absolute birth weight muddles growth restriction with prematurity.

4. In line 204 the authors write that while the rate of prematurity in VT is same as singletons, there is more LBW < 2500g. However, that statistic compares 3 groups (twins, VTS, singleton and <0.001 however the twin percentage is 29% and this may be driving the p value to significant. Is the 19% VTS different than the 14.4% in singletons (wondering if the p value represents the difference between twins and singletons only?)

5. Is it possible for the authors to explore if using a three group comparison statistic is ideal ? For example, in lines 213 the authors perform direct comparisons and it is more informative (VTS to singleton and VTS to twins). Is it possible to

compare VTS to singleton, and VTS to twins separately and consistently throughout the text?

6. Currently some tables have VTS as the first column and others have VTS as second column, this is confusing please standardize

7. The placental descriptions are very detailed, and the authors do make an effort to introduce the concepts in the introduction which is helpful. However, in lines 220-223 I found the wording cumbersome and I was getting lost, perhaps because the authors are describing a difference in VTS patients but the p values are 0.76 (NS)?. Its great the authors provided Table 2 in recognition that many readers may not be familiar with the significance of all these categories. Perhaps in the text, a more concise approach can be taken to describing the pathology perhaps using the sub-categories presented in table 3 such as "any vascular" or "any inflammatory"? The authors should only describe results in the text that they intend to later explain and address in the comments.

8. Could the authors comment on what is the possible explanation why twins should have less evidence of infectious pathology than singletons or VTS?

STATISTICAL EDITOR COMMENTS:

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

lines 59-60, 178-180: There is no trend, the association was rejected via a priori definition of statistical significance.

Table 1: The number of embryos transferred can only have integer values, similar to parity. Should cite as median (IQR or range) or as categories.

All Tables: The Vanishing twin cohort has n =73, so the n(%) should be formatted as n(integer %), not as 0.1% precision.

Table 2: Is GA cited as mean? Should state.

Tables 3, 4: There are 5 variables used as adjustors in these aORs. The counts, esp among vanishing twins vs twins are too few for infectious or inflammatory pathologies to allow for multiple adjustment. Likely those aOR models are over fitted.

Suppl Table 3: The n=20 for vanishing twin > 8 wk and the individual row categories are subsets of that number. Therefore, the comparisons vs singletons, although all NS, are very likely under powered.

EDITOR'S COMMENTS:

We no longer require that authors adhere to the Green Journal format with the first submission of their papers. However, any revisions must do so. I strongly encourage you to read the instructions for authors (the general bits as well as those specific to the feature-type you are submitting). The instructions provide guidance regarding formatting, word and reference limits, authorship issues, and other 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.

PRESENTATION OF STATS INFORMATION
P Values vs Effect Size and Confidence Intervals
While P values are a central part of inference testing in statistics, when cited alone, often the strength of the conclusion can be misunderstood. Whenever possible, 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.
This is true for the abstract as well as the manuscript, tables and figures.

Please provide absolute values for variables, in addition to assessment of statistical significance.

We ask that you provide crude OR's followed by adjusted OR's for all relevant variables.

Please avoid causal language throughout your manuscript. Your study can identify and quantify associations, but not causation. Language should be changed in the precis, abstract, and manuscript, if causal language is used in those sites.

Line 37: The objective of the abstract should be a simple “To” statement without background information.

Lines 58: Please also provide comparison to twin pregnancies as twin pregnancies are at increased risk of many of these placental findings. Without this comparison, one cannot conclude that it’s the VTS and not the initial twinning that is associated with the abnormal placentas. This also affects your precis.”

Also, for the readership of the journal, it is important for you to focus more on the ob outcomes as we are primarily a clinical journal. The abstract should provide data about these outcomes, and your discussion could reasonably focus much more on those.

Line 59: We do not allow authors to describe variables or outcomes in terms that imply a difference (such us of the terms “trend” or “tendency” or “marginally different”) unless there is a statistical difference. Please edit here and throughout to indicate that there is no difference.

Line 65: It is an idiosyncratic fact that at the Journal we tend to avoid the use of the word impact to imply the result of a change, preferring to limit "impact" to mean a physical blow.

Line 67: I don’t know what you mean by “Vanishing twin syndrome conceived through IVF”. Perhaps “Following IVF, singleton pregnancies resulting from vanishing twin syndrome had similar outcomes as singleton conceptions”

Your introduction is quite long and much of the content should be moved to the discussion. We recommend limiting the introduction to approximately one page.

Line 102: is this in reference to the placenta of the surviving twin? The placenta of the dead twin wouldn’t have fetal vascular malperfusion as there would be NO perfusion.

Line 105: Please edit out the “to our knowledge” or similar wording. As the readers cannot gauge the depth and breadth of your knowledge, this phrase does not add significant meaning. You can either reference your literature search details (database searched and search terms used) that informed your knowledge, or you could say something noting that your cited references provide limited information about this point.

This sentence also gets confusing about whether you are writing about the dead twin or the surviving twin’s placenta. Please clarify.

Line 112: again, are you trying to get at placental changes in the survivor or dead twin?

Lines 124-136: could you make the IVF management methods section a bit more concise? For instance, we can assume that a fertility evaluation was done and that you monitored follicular development by US. If there are details that might influence rate of twinning, they should be retained.

Line 151: Please edit to “fetal growth restriction”

Please include in your analysis those cases confirmed to be monozygotic based on twinning after single embryo transfer. Outcomes of MZ twins differ from DZ twins. I realize that this will get to pretty small numbers but this needs to be included at least as descriptive data

Section on placental examinations: Please indicate that when placenta from VTS pregnancy is described that this excluded any remnant of the demised twin’s placenta and the you are ONLY describing results of the surviving twin’s placenta.

Line 184: Please note that your study was conducted from date 1 to date 2, not between those dates. As written, it would exclude the dates given .

Line 185: One line 159 you indicated that IVF conception was an indication for full placental examination but here, fewer than 1/3 actually had placental pathology done. This is a limitation that will need to be addressed in your discussion.

Line 187: In discussion, please offer an explanation for why your VTS rate is < ½ of what you indicated was the typical rate after twin conception.

Line 188: Do not begin a sentence with a numeral. Either spell out or edit your sentence to avoid the need to start w/ a number.

Line 199: as noted by statistical editor, # of embryos should be reported as interquartile ranges as you cannot transfer a part of an embryo.

In your results, please make sure you present the data for VTS, singleton and twin pregnancies and always in the same order to avoid confusion

Line 211-214: Please provide comparative results and statistical evidence to support assertion of differences between groups. So for instance, what was the rate of membranous cord insertion (Do you mean velamentous?) and accessory placental lobes in twins and singletons?

Line 215: Our placental pathologist at UNC does not assume an infectious etiology for all cases of chorioamnionitis on pathology. Does yours? Labor does influence the finding of inflammation—it could be that since your CS rate in twins (likely many of them pre-labor) is higher than in your singleton deliveries, the difference may be due to labor v no labor.

Line 218, 221,223. 243: We do not allow authors to describe variables or outcomes in terms that imply a difference (such us of the terms “trend” or “tendency” or “marginally different”) unless there is a statistical difference. Please edit here and throughout to indicate that there is no difference.

Line 227: Please define placental bed disruption

Line 231: Please provide data for comparison of VTS to twins.

Lines 232-235: please clarify. What is the difference between “infectious pathology “ and “Inflammatory pathology”?

Line 239: Please also provide comparisons to twins.

Line 248…”affects placental development of the surviving twin”.

Line 270+: There was no difference so you can’t describe them here as different.

Line 274: This emphasizes the importance of distinguishing, in your methods section, between “inflammation” and “chorioamnionitis”, which is an inflammatory process.

Line 278: The journal style does not support the use of the virgule (/) except in mathematical expressions. Please remove here and elsewhere.

Line 301: This is known as a primacy claim: yours is the first, biggest, best study of its kind. In order to make such a claim, please provide the data bases you have searched (PubMED, Google Scholar, EMBASE for example) and the search terms used. IF not done, please edit it out of the paper.

Line 303: In here, please discuss the relatively low rate (28%) of placental examinations of eligible patients as this may lead to an ascertainment bias.

EDITOR COMMENTS:

1. The Editors of Obstetrics & Gynecology are seeking to increase transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:
A. OPT-IN: Yes, please publish my point-by-point response letter.
B. OPT-OUT: No, please do not publish my point-by-point response letter.

2. 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.

Please check with your coauthors to confirm that the disclosures listed in their eCTA forms are correctly disclosed on the manuscript's title page.

3. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript's lead author. The statement is as follows: "The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained." *The manuscript's guarantor.

If you are the lead author, please include this statement in your cover letter. If the lead author is a different person, please ask him/her to submit the signed transparency declaration to you. This document may be uploaded with your submission

in Editorial Manager.

4. In order for an administrative database study to be considered for publication in Obstetrics & Gynecology, the database used must be shown to be reliable and validated. In your response, please tell us who entered the data and how the accuracy of the database was validated. This same information should be included in the Materials and Methods section of the manuscript.

5. All studies should follow the principles set forth in the Helsinki Declaration of 1975, as revised in 2013, and manuscripts should be approved by the necessary authority before submission. Applicable original research studies should be reviewed by an institutional review board (IRB) or ethics committee. This review should be documented in your cover letter as well in the Materials and Methods section, with an explanation if the study was considered exempt. If your research is based on a publicly available data set approved by your IRB for exemption, please provide documentation of this in your cover letter by submitting the URL of the IRB website outlining the exempt data sets or a letter from a representative of the IRB. In addition, insert a sentence in the Materials and Methods section stating that the study was approved or exempt from approval. In all cases, the complete name of the IRB should be provided in the manuscript.

6. Supplemental Table 2 - This appears to be reprinted from a previous publication by the first author. Please provide a letter of permission for electronic use.

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 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.

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 22 typed, double-spaced pages (5,500 words). Stated page limits include all numbered pages in a manuscript (i.e., title page, précis, abstract, text, references, tables, boxes, figure legends, and print appendixes) but exclude references.

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, including spaces, for use as a running foot.

11. 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.

12. 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.

13. 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.

14. 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%").

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

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 through Editorial Manager at http://ong.editorialmanager.com. Your manuscript should be uploaded in a word processing format such as Microsoft Word. Your revision's cover letter should include the following:

* A confirmation that you have read the Instructions for Authors (http://edmgr.ovid.com/ong/accounts/authors.pdf), and
* A point-by-point response to each of the received comments in this letter.

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

Again, your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Feb 21, 2020, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Nancy C. Chescheir, MD
Editor-in-Chief

2018 IMPACT FACTOR: 4.965
2018 IMPACT FACTOR RANKING: 7th out of 83 ob/gyn journals

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