OBSTETRICS & GYNECOLOGY

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

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

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

Date: Dec 13, 2019
To: "Michael Blake Evans" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-19-2135

RE: Manuscript Number ONG-19-2135

MATURE FOLLICLE COUNT AND MULTIPLE GESTATION RISK BASED ON PATIENT AGE IN INTRAUTERINE INSEMINATION CYCLES WITH OVARIAN STIMULATION

Dear Dr. Evans:

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 Jan 03, 2020, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1: In this manuscript, the authors present a study of IUI with ovarian stimulation relying on a large sample from a private fertility clinic (Shady Grove Fertility). The study seeks to identify how age impacts the risk of multiples as the number of follicles increases in a given cycle. This study question is relevant. Avoiding multiples is ideal and, as noted by the authors, increasingly recognized as a quality indicator. That said, in the IUI setting, there are no options besides canceling a cycle if there is a concern for multiple gestations. When is too many follicles too many and how does age have an effect on that question. This was the gist of this study. Overall, the sample is large and the analysis is appropriate to answer the study question. I have the following specific questions/comments:

1) Is this topic too specific for a journal focused on general OB/GYN practice? I could go both ways - if specialists are doing IUI, then this paper is highly practical and could be useful in their practice. That said, how many specialists do IUI?

2) The introduction could be shorter. Combine the 1st and 3rd paragraphs - both have to do w/ ovarian stimulation - and start the introduction w/ a thinner 2nd paragraph.

3) Methods - start w/ "this study was done at Shady Grove…" It's much more orienting to think about the methods as starting w/ a place and moving to what you did in that place. I think it would be also helpful to say a bit about Shady Grove. It is mentioned that this is a private practice in the abstract but not in the methods.

4) Is it possible that over the 13 years practice styles changed? Would that matter? I'm betting this is all highly protocolized medicine so was there a protocol?

5) How were the data used in this study a) stored at Shady Grove and b) collected for research? If there was a protocol it would seem there should have been a standard set of labs and documentation regarding each cycle. How was this stored? Was there a difference in that storage over the 13 years? If so, how did that get accounted for in assembling the research dataset? Seems like this sort of questioning has relevance to some of the admitted study weaknesses. Were follicles <14mm not counted in the records? Were not all the subjects infertility diagnoses not recorded?

6) LOVE the heatmap!

Overall, interesting and helpful study.

Reviewer #2: The authors have tackled an exceptionally important issue and it is commendable that they are drawing

upon what may be the single largest database to address this question. They simply present their data, and given that a future publication with a larger sample size to address the question is improbable until widespread individual patient meta-analysis becomes common, this paper will be the definitive article on the subject for the foreseeable future. Moreover, as Adashi's NEJM article showed, there is far more opportunity to reduce high order multiple gestation through superovulation than there is with IVF, particularly in an era of SET after PGT-A.

That being said, the authors have not adequately addressed meaningful confounding by indication in their study, particularly with regard to anovulatory patients and the duration of subfertility. Multiple authors from Guzick to Bhattacharya have shown that insemination in isolation offers limited benefit to fertility (outside of severe male factor, which was excluded for this study). If monofollicular recruitment is performed using any medication for an ovulatory patient, then the sole benefit is insemination, which minimally shifts fecundity. (Literature published on unexplained infertility does not typically describe 14% monthly fecundity under 38 years with simple insemination for Figure 1B. Addressing why this population may differ is critical.) One exception for this is anovulatory patients, where going from zero oocytes available in a month to one makes an enormous difference in the odds of conception (relative to going from one to two). These are arguably the patients most likely to conceive when oral and injectable medications are introduced and have the greatest risk for multiple gestation, with Kate Gosselin being the poster-child.

Arguably, many cancel months for insemination if there is monofollicular recruitment in ovulatory patients and simply wait for another month with a more robust response. If the monofollicular data is driven by oligo or anovulatory patients (and also use of donor sperm for same sex or single women, which wasn't clearly excluded under the definition of severe male factor), while the multifollicular data is driven by ovulatory patients, then this has meaningful implications for the efficacy of therapy. This is why when looking at unexplained infertility alone, dual follicular recruitment was 66% more effective than when looking at all categories (AOR 1.3 ≠ 1.5), as one has lost how anovulatory patients bias the efficacy of multifollicular recruitment towards the null. Conversely, it seems counterintuitive that the AOR for multiples is unchanged when contrasting all categories with unexplained infertility. (Or, if we should stop thinking of anovulatory status as increasing risk for multiple gestation, this needs to be addressed. Is the lack of difference in the AOR for unexplained infertility a function of the absolute risk of multiple gestation being lower relative to all categories?)

Another area where there can be meaningful confounding for both fecundity and multiple gestation risk relates to duration of subfertility. Patients just starting on this journey (such as same sex couples performing donor insemination) have higher chances of pregnancy, as well as greater risk for multiples if multifollicular recruitment occurs (such as when some take oral medication to accelerate fertility owing to the cost of donor sperm, even though this is much higher risk). Superovulation with three oocytes for a couple just starting their procreative journey is very different from those with eight years of infertility, where adding another three oocytes isn't quite as risky for multiples (or pregnancy itself) when the previous ninety-six have failed.

The issue of inherent prognosis goes beyond these examples. For example, tubal disease is heterogeneous, where superovulation for a patient who has just had tubal reversal has a very different prognosis for fecundity and multiples relative to a patient with persistent bilateral hydrosalpinges after neosalpingostomies.

There is a tendency among reviewers to believe that, "If there is data, let's go with the data. If there are opinions, let's go with mine." The authors draw upon an exceptionally strong database, but as a clinician who has performed several thousand inseminations over the past few decades with two to three follicles (and not unfrequently four for women over 35, where arguably half of oocytes may be aneuploid) without a single high order pregnancy, this paper doesn't pass the sniff test when the authors state, "The risk of triplets is as high as 2% to 8% among patients under 41 years with 3 to 5 follicles." I don't believe myself to be uniquely lucky, but rather that the gap likely lies in how we account for factors such as ovulatory status and duration of subfertility.

In short, this is a very important paper, but as currently written would be misleading without properly controlling for confounding by indication. Not only should the data be better substratified, but the authors must address the interplay of multifollicular recruitment on fecundity and multiples relative to patient prognosis. Caution is necessary to minimize multiples, but this must be balanced with setting a standard that would promote less effective care in those needing it most.

Reviewer #3:

Thank you for the opportunity to review the manuscript. Evans et al. in their study have looked into a very clinically relevant question. The manuscript is well written, flows well and is supported by some excellent graphical representation which may help in patient counselling. However, the manuscript contains some significant drawbacks (as the authors have stated in the discussion section).

Please find my thoughts below:

Aspect 1 - The indication for OI and IUI treatment is not mentioned.
Aspect 2 - Duration of infertility.
Aspect 3 - Type of medications used and dosing regimen are missing.

All the above parameters are important when we counsel women about treatment option and sadly, these are all missing.

Aspect 4 - Methods section should be more elaborate. For e.g:

What day of treatment cycle was the ultrasound performed ?
What was the criteria for ovulation trigger?
What dose of hCG was used - low dose hCG versus high dose hCG based on BMI cut off?

Aspect 5 - A year by year analysis of multiple rates may be beneficial given missing parameters mentioned in aspect 1. This is because previously clomid may have been used more frequently for PCOS. More recently, guidelines suggest using Letrozole, which helps in mono-follicular recruitment.

Aspect 6 - Authors mention a very strict cut off for insemination based on sperm parameters. Given all the parameters, this probably was an excellent cohort of patients who may have got pregnant with a more conservative approach.

Aspect 7 - In the discussion section, can the first line of the paragraph be a summary of the study finding ?

STATISTICAL EDITOR'S COMMENTS:

1. lines 70-71: The odds (adjusted odds) were tripled, not the likelihood.

2. lines 156-159: Should include in Results the number of women, along with the number of IUI cycles and the median (range) of cycles per woman.

3. lines 162-164: Should define what is meant by "negligibly", since the relative increase was ~ 16% and those proportions are significantly different by Chi-square (p = .03), although not as large as the differences in rates of twins or higher order multiples.

4. lines 179-180: Should define "stable". This comparison, using data from Table 1, is NS by Chi-square.

5. Fig 2: Should include CIs for the risk estimates.

6. Fig 3: The chart could be misinterpreted by patients, since it does not include CIs for each of the estimates.

EDITORIAL OFFICE COMMENTS:

1. The Editors of Obstetrics & Gynecology are seeking to increase transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:
A. OPT-IN: Yes, please publish my point-by-point response letter.
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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. Was this study presented at ASRM in 2018? If so, please disclose the name, date/s, and location of the meeting on the title page of your manuscript.

4. 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.

5. 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.

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

7. 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).

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

9. 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: Current Commentary articles, 250 words. Please provide a word count.

10. 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.

11. 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.

12. 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%").

13. 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.

14. 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.

15. The Journal's Production Editor had the following comments about the figures in your manuscript:

"Figure 1: Please fill the bars with a solid color, rather than a gradient.
Figure 2: Please fill the bars with a solid color, rather than a gradient. "

When you submit your revision, art saved in a digital format should accompany it. If your figure was created in Microsoft Word, Microsoft Excel, or Microsoft PowerPoint formats, please submit your original source file. Image files should not be copied and pasted into Microsoft Word or Microsoft PowerPoint.

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

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

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

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

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

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 Jan 03, 2020, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

The Editors of Obstetrics & Gynecology

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

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DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

[redacted]

M. Blake Evans, D.O.
[redacted]

Nancy C. Chescheir, MD
Editor-in-Chief, Obstetrics and Gynecology
409 12th Street, SW
Washington, DC 20024

January 17, 2020

Dear Dr. Chescheir;

Attached is the revision of the manuscript addressing all inquiries. Thank you for the opportunity to submit our manuscript entitled, “Mature Follicle Count and Multiple Gestation Risk Based On Patient Age In Intrauterine Insemination Cycles With Ovarian Stimulation” to Obstetrics and Gynecology. This retrospective cohort of 50,473 intrauterine insemination cycles evaluates the risk of a multiple gestation in ovarian stimulation intrauterine insemination cycles with regard to patient age and follicle number. Clinically useful graphs have been made that can be utilized to counsel the patient on what her overall clinical pregnancy rate is, absolute multiple risk, and if she became pregnant, what her risk of multiples would be. The figures serve as tools that can be used clinically to show patients visual estimates of their risks and benefits. Results from this study show that caution should be used in ovulation induction with > 2 mature follicles in women under the age of 40, as further follicular development substantially increases the risk of multiple gestation without an improved chance of clinical pregnancy. Up to 4 follicles improves pregnancy rates in women over 40 without substantially increasing the risk of multiples.

All authors have reported if they had any conflicts of interest and permission has been obtained to submit the manuscript.

Preliminary data of this study was presented at the Armed Forces District meeting in Honolulu, HI, September 25th, 2018 and the American Society of Reproductive Medicine conference in October 8th, 2018 in Denver, CO entitled: “Mature follicle count and multiple gestation risk based on patient age in ovulation induction-intrauterine insemination (OI-IUI) cycles.” The manuscript is not under consideration for another journal, and is unique. All authors have read and approved the manuscript for

submission. The study was performed at Shady Grove Fertility Center in Rockville, Maryland, with Institutional Review Board approval.

Thank you for your consideration.

Author contributions:
MBE constructed original idea, reviewed literature, collected data, performed data analysis, made the figures/tables and wrote the manuscript.
BS reviewed literature, and wrote the manuscript.
KSR collected data, reviewed the manuscript, and helped make figures.
MC, EW, MWH, RS, and AHD reviewed the manuscript.
NCS, KD, and MJH performed data analysis, and wrote the manuscript.

Author Declaration of Transparency: The lead author, MBE 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 have been explained.

Sincerely,

M. Blake Evans, DO, on behalf of the authors

REVIEWER COMMENTS:

Reviewer #1: In this manuscript, the authors present a study of IUI with ovarian stimulation relying on a large sample from a private fertility clinic (Shady Grove Fertility). The study seeks to identify how age impacts the risk of multiples as the number of follicles increases in a given cycle. This study question is relevant. Avoiding multiples is ideal and, as noted by the authors, increasingly recognized as a quality indicator. That said, in the IUI setting, there are no options besides canceling a cycle if there is a concern for multiple gestations. When is too many follicles too many and how does age have an effect on that question. This was the gist of this study. Overall, the sample is large and the analysis is appropriate to answer the study question. I have the following specific questions/comments:

1) Is this topic too specific for a journal focused on general OB/GYN practice? I could go both ways - if specialists are doing IUI, then this paper is highly practical and could be useful in their practice. That said, how many specialists do IUI?

Thank you for your question. We feel that this is an extremely important topic that is applicable to anyone performing ovulation induction-IUIs. (REIs, OBGYN specialists, Nurse Practitioners, Physician Assistants, etc.) and would benefit having this information to guide safe patient care. We felt that a broad and highly read journal such as Obstetrics and Gynecology would be a fantastic platform to widely distribute this data and reduce the risk of multiples.

2) The introduction could be shorter. Combine the 1st and 3rd paragraphs - both have to do w/ ovarian stimulation - and start the introduction w/ a thinner 2nd paragraph.

Pages 1-2:
The second paragraph condensed and moved to the start of the introduction. 1st and 3rd paragraphs condensed and merged.

3) Methods - start w/ "this study was done at Shady Grove…" It's much more orienting to think about the methods as starting w/ a place and moving to what you did in that place. I think it would be also helpful to say a bit about Shady Grove. It is mentioned that this is a private practice in the abstract but not in the methods.

Comment addressed in methods section:

Page 6:
All OS-or OI-IUI cycles were performed at Shady Grove Fertility Center; a private practice fertility center in Rockville, MD

4) Is it possible that over the 13 years practice styles changed? Would that matter? I'm betting this is all highly protocolized medicine so was there a protocol? .

There was no change in protocols over that time period. Methods updated to address your comment:

Page 6-7:
OS and OI protocols did not change during the study period. Ovulation induction medications were utilized per the discretion of the provider. Letrozole (2.5-7.5 mg), clomiphene citrate (50-150 mg), recombinant follitropin or menotropin (75 international units) were initiated on cycle day 3 after a baseline pelvic ultrasound was performed, and taken daily for 5 days unless inadequate follicle response. If gonadotropins were supplemented to either clomiphene or letrozole cycles, 75 international units of follitropin or menotropin were added on cycle days 8 to 10, after completion of a 5 day regimen of an oral agent. A follicle scan was performed between cycle day 9 and 12, and then every 1 to 3 days as needed until the lead follicle reached 18-20 mm in greatest diameter. All patients received recombinant human chorionic gonadotropin injections (250 microgram subcutaneous or 10,000 units intramuscular per provider preference) when the lead follicle was 18-20mm If a serum luteinizing hormone was obtained and a surge was noted to have occurred (> 20 international units), the trigger was omitted. The BMI cut off was above 44 kilogram per meter^2.

5) How were the data used in this study a) stored at Shady Grove and b) collected for research? If there was a protocol it would seem there should have been a standard set of labs and documentation regarding each cycle. How was this stored? Was there a difference in that storage over the 13 years? If so, how did that get accounted for in assembling the research dataset? Seems like this sort of questioning has relevance to some of the admitted study weaknesses. Were follicles <14mm not counted in the records? Were not all the subjects infertility diagnoses not recorded?

Thank you for the questions/comments. All data is stored into ArtWorks by designated nurses that work with each provider. We began clinical use of our current EMR (ArtWorks) at Shady Grove in 2002, so the data input/collecting methods have been similar over the years. The protocol information has been entered into the methods section and did not change over the study period.

The following were added to the Methods to address the questions:
Page 6:
All clinical data were stored in the same electronic medical record system during the study period. The same clinical variables were collected over the entire period of the study. The electronic medical record was queried to capture all IUI cycles and the extract the desired variables for the research study.

Page7:
Data was missing on infertility diagnosis for 2,264 cycles (4.4%). These cycles were included in the overall study analysis but not the subgroup analysis of unexplained infertility and anovulation.

Page 8:
The total number of follicles ≥ 14 mm was recorded as a field in each patient's electronic medical record over the entire study period. Follicles <14mm were not recorded in the majority of patient records.

6) LOVE the heatmap!

We are pleased that you like it. We truly think that this will be a valuable tool for both physicians during counselling and help patients visually understand the risks vs benefits.

Overall, interesting and helpful study.

Reviewer #2: The authors have tackled an exceptionally important issue and it is commendable that they are drawing upon what may be the single largest database to address this question. They simply present their data, and given that a future publication with a larger sample size to address the question is improbable until widespread individual patient meta-analysis becomes common, this paper will be the definitive article on the subject for the foreseeable future. Moreover, as Adashi's NEJM article showed, there is far more opportunity to reduce high order multiple gestation through superovulation than there is with IVF, particularly in an era of SET after PGT-A.

That being said, the authors have not adequately addressed meaningful confounding by indication in their study, particularly with regard to anovulatory patients and the duration of subfertility. Multiple authors from Guzick to Bhattacharya have shown that insemination in isolation offers limited benefit to fertility (outside of severe male factor, which was excluded for this study). If monofollicular recruitment is performed using any medication for an ovulatory patient, then the sole benefit is insemination, which minimally shifts fecundity. (Literature published on unexplained infertility does not typically describe 14% monthly fecundity under 38 years with simple insemination for Figure 1B. Addressing why this population may differ is critical.) One exception for this is anovulatory patients, where going from zero oocytes available in a month to one makes an enormous difference in the odds of conception (relative to going from one to two). These are arguably the patients most likely to conceive when oral and injectable medications are introduced and have the greatest risk for multiple gestation, with Kate Gosselin being the poster-child.

Arguably, many cancel months for insemination if there is monofollicular recruitment in ovulatory patients and simply wait for another month with a more robust response. If the monofollicular data is driven by oligo or anovulatory patients (and also use of donor sperm for same sex or single women, which wasn't clearly excluded under the definition of severe male factor), while the multifollicular data is driven by ovulatory patients, then this has meaningful implications for the efficacy of therapy. This is why when looking at unexplained infertility alone, dual follicular recruitment was 66% more effective than when looking at all categories (AOR 1.3 ◊ 1.5), as one has lost how anovulatory patients bias the efficacy of multifollicular recruitment towards the null. Conversely, it seems counterintuitive that the AOR for multiples is unchanged when contrasting all categories with unexplained infertility. (Or, if we should stop thinking of anovulatory status as increasing risk for multiple gestation, this needs to be addressed. Is the lack of difference in the AOR for unexplained infertility a function of the absolute risk of multiple gestation being lower relative to all categories?)

We appreciate the reviewer's comments, which get at the core of a clinical paradigm: the suggestion that ovarian stimulation should be used for mono-follicular development in anovulatory patients and multi-follicular development for patients with non-ovulatory infertility. We did further subanalyses to look at anovulatory patients to investigate this deeper. The findings interestingly demonstrate the paradigm to perhaps hold, given that the increase RR of clinical pregnancy is greater with each additional follicle for UI and the increase in the RR of multiple gestation with each additional follicle is higher for multiple gestation in the anovulatory group. However, the results also challenge this paradigm, as the RR with more follicles in each group is much greater for multiple gestation risk than for increasing pregnancy.

Pages 12-13:

Ovulatory Disorders:

Additional analyses were done on patients with polycystic ovarian syndrome (PCOS) or oligo-ovulation (20.1%, n = 10,089 cycles). 36.7% of these IUI cycles utilized clomiphene citrate alone, 30.0% used gonadotropins alone, 23.0% used gonadotropins plus clomiphene, 8.5% used letrozole alone, and the remainder utilized letrozole plus gonadotropins. When limiting to women with ovulatory disorders, all age groups revealed a similar trend to the overall cohort with increased chance of clinical pregnancy and multiple pregnancy with each additional follicle. In all age groups with ovulatory disorders, increasing the number of mature follicles from 1 to 5 had a similar increase in clinical pregnancy: 2 follicles compared to 1 (AOR 1.3, CI 1.1-1.4), 3 follicles compared to 1 (AOR 1.5, CI 1.3-1.7), 4 follicles compared to 1 (OR 1.8, CI 1.5-2.1), and 5 follicles compared to 1 (AOR 1.6, CI 1.2-2.1). However, all age groups also revealed a significantly increased risk of multiples with

each increasing mature follicle number: with 2 follicles compared to 1 (AOR 3.9, 95% CI 2.5-6.0), 3 follicles compared to 1 (AOR 8.2, 95% CI 5.2-12.7), 4 follicles compared to 1 (AOR 12.5, 95% CI 7.7-20.5) and 5 follicles compared to 1 (AOR 14.3, 95% CI 7.7-26.7).

Page 16:

Clinical paradigms in managing ovarian stimulation historically have been to induce mono-follicular development in anovulatory patients versus trying to induce multi-follicular development in patients with unexplained infertility. Subgroup analyses demonstrated that multifollicular development resulted in higher odds of clinical pregnancy in patients with unexplained infertility but also resulted in a higher odds of multiple gestation in anovulatory patients. While these result support the historical paradigm, it should be noted that the 95%CI of most of these estimates overlapped, precluding a definitive conclusion. However, in both groups the increased odds of multiple gestation were greater than the increased odds of clinical pregnancy. This suggests that caution should be employed when considering IUI in all patients with more than two follicles, regardless of the diagnosis, if the goal is to achieve a singleton pregnancy.

Another area where there can be meaningful confounding for both fecundity and multiple gestation risk relates to duration of subfertility. Patients just starting on this journey (such as same sex couples performing donor insemination) have higher chances of pregnancy, as well as greater risk for multiples if multifollicular recruitment occurs (such as when some take oral medication to accelerate fertility owing to the cost of donor sperm, even though this is much higher risk). Superovulation with three oocytes for a couple just starting their procreative journey is very different from those with eight years of infertility, where adding another three oocytes isn't quite as risky for multiples (or pregnancy itself) when the previous ninety-six have failed.

We found no association of duration of infertility with longer duration of infertility.

Page 16

Another clinical paradigm is that the duration of infertility justifies the stimulation of more mature follicles. When evaluating duration of infertility as a continuous variable and as a categorical variable (<12, 12-23, 24-35, and ≥36 months) and adjusting for patient age, duration of infertility was not associated with either clinical pregnancy or multiple gestation. These data suggest caution should be used in aggressive ovarian stimulation based on duration on infertility.

The issue of inherent prognosis goes beyond these examples. For example, tubal disease is heterogeneous, where superovulation for a patient who has just had tubal reversal has a very different prognosis for fecundity and multiples relative to a patient with persistent bilateral hydrosalpinges after neosalpingostomies.

We agree with this point, which is a weakness of most studies on infertility. We categorized our data into broad diagnostic groups similar to what is required in SART reporting and is standard practice. Dividing subgroups, such as tubal infertility, into all the dozens of potential etiologies was not practical and would reduce either the power to detect meaningful conclusions or result in hundreds of comparisons. We have added your point to the discussion on weaknesses.

Page 16:

Another weakness is that infertility was grouped according to broad diagnoses categories, which may represent a heterogenous group of patients with a wide range of prognosis for fecundity and multiple gestation. Uterine factor and tubal factor infertility are two examples of broad diagnoses categories that would have a wide range of disease states. We did not subdivide infertility groups into smaller specific etiologies, as the number of categories would become very large and power would be lost to detect meaningful differences.

There is a tendency among reviewers to believe that, "If there is data, let's go with the data. If there are opinions, let's go with mine." The authors draw upon an exceptionally strong database, but as a clinician who has performed several thousand inseminations over the past few decades with two to three follicles (and not unfrequently four for women over 35, where arguably half of oocytes may be aneuploid) without a single high order pregnancy, this paper doesn't pass the sniff test when the authors state, "The risk of triplets is as high as 2% to 8% among patients under 41 years with 3 to 5 follicles." I don't believe myself to be uniquely lucky, but rather that the gap likely lies in how we account for factors such as ovulatory status and duration of subfertility.

This sentence was modified.

In short, this is a very important paper, but as currently written would be misleading without properly controlling for confounding by indication. Not only should the data be better substratified, but the authors must address the interplay of multifollicular recruitment on fecundity and multiples relative to patient prognosis. Caution is necessary to minimize multiples, but this must be balanced with setting a standard that would promote less effective care in those needing it most.

We greatly appreciate the thoughtful comments from Reviewer 2 and feel the subanalyses suggested have added to the value of the paper. Indeed, the data support why traditional paradigms exist but more importantly challenge the practice of trying to stimulate more than 2 follicles in IUI cycles. While the multiple gestation rate or triplet rate may seem high compared to this expert reviewer's personal experience, they represent real world data from over 50,000 cycles. We believe this reviewer's suggestions have challenged us to interrogate the data at a deeper level and revealed some interesting outcomes that challenge some common practices.

Reviewer #3:

Thank you for the opportunity to review the manuscript. Evans et al. in their study have looked into a very clinically relevant question. The manuscript is well written, flows well and is supported by some excellent graphical representation which may help in patient counselling. However, the manuscript contains some significant drawbacks (as the authors have stated in the discussion section).

Please find my thoughts below:

Aspect 1 - The indication for OI and IUI treatment is not mentioned.

We have added more detail to the paper to address comments 1-4

Pages 6-7:
Ovulation induction medications were utilized per the discretion of the provider. Letrozole (2.5-7.5 mg), clomiphene citrate (50-150 mg), recombinant follitropin or menotropin (75 international units) were initiated on cycle day 3 after a baseline pelvic ultrasound was performed, and taken daily for 5 days unless inadequate follicle response. If gonadotropins were supplemented to either clomiphene or letrozole cycles, 75 international units of follitropin or menotropin were added on cycle days 8 to 10, after completion of a 5 day regimen of an oral agent. A follicle scan was performed between cycle day 9 and 12, and then every 1 to 3 days as needed until the lead follicle reached 18-20 mm in greatest diameter. All patients received recombinant human chorionic gonadotropin injections (250 microgram subcutaneous or 10,000 units intramuscular per provider preference) when the lead follicle was 18-20mm If a serum luteinizing hormone was obtained and a surge was noted to have occurred (> 20 international units), the trigger was omitted. The BMI cut off was above 44 kilogram per meter^2.

Aspect 2 - Duration of infertility.

Page 7:
Duration of infertility was defined as the number of reported months without contraception while being sexually active. The relationship of duration of infertility with clinical pregnancy and multiple gestation was assessed by subgroups (<12 months, 12-23 months, 24-25 months, and ≥36 months) and as a continuous variable.

Aspect 3 - Type of medications used and dosing regimen are missing.

See above reply #1

All the above parameters are important when we counsel women about treatment option and sadly, these are all missing.
Thank you for these comments and the other reviewer who suggested the same.

Aspect 4 - Methods section should be more elaborate. For e.g:

What day of treatment cycle was the ultrasound performed?

premed day 3; first follicular ultrasound between day 9 and 12 then q 1-3 days until 18-20mm follicle present. This information is now in the methods.

What was the criteria for ovulation trigger?
Largest follicle >/= 18mm if gonadotropins were used and >/= 20 mm if oral meds only. If LH surge (generally>20) noted then trigger generally omitted. This has been added to thte methods.

What dose of hCG was used - low dose hCG versus high dose hCG based on BMI cut off?
rec HCG (e.g. ovidrel) for everyone. BMI cutoff 44. Please see revision.

Aspect 5 - A year by year analysis of multiple rates may be beneficial given missing parameters mentioned in aspect 1. This is because previously clomid may have been used more frequently for PCOS. More recently, guidelines suggest using Letrozole, which helps in mono-follicular recruitment.

We evaluated year of treatment and found no relationship with the outcomes, likely given the relatively stable nature of stimulation protocols

Page 7:
There was no difference in clinical pregnancy or multiple gestation based on duration of infertility. Year of treatment was assessed to examine if practice pattern changes (for example more letrozole use in later years) was associated with differences in clinical pregnancy or multiple gestation. However, these outcomes did not change over the study period, indicating the relatively stable outcome results in IUI cycles over the study period.

Aspect 6 - Authors mention a very strict cut off for insemination based on sperm parameters. Given all the parameters, this probably was an excellent cohort of patients who may have got pregnant with a more conservative approach.

Thank you for the comment. I have added this to a limitation in the discussion. Based off of prior internal data, as cited in the discussion, indicates that pregnancy rates were stable over 8 million TMS in IUI cycles. Yes, this may include many patients that are good prognosis, but we also wanted to include a large range of patients that benefit from IUI (ie, oligospermia < 15 million/mL per WHO criteria), but are also not so oligospermic that they would be considered for IVF and have quite a low prognosis for IUI.

Aspect 7 - In the discussion section, can the first line of the paragraph be a summary of the study finding ?

Page13:
This large retrospective study reveals that caution should be used in ovulation induction with greater than 2 mature follicles in women under the age of 40, as further follicular development substantially increases the risk of multiple gestation without an improved chance of clinical pregnancy.

STATISTICAL EDITOR'S COMMENTS:

1. lines 70-71: The odds (adjusted odds) were tripled, not the likelihood.
Revised

2. lines 156-159: Should include in Results the number of women, along with the number of IUI cycles and the median (range) of cycles per woman.
Revised

3. lines 162-164: Should define what is meant by "negligibly", since the relative increase was ~ 16% and those proportions are significantly different by Chi-square (p = .03), although not as large as the differences in rates of twins or higher order multiples.
Revised

4. lines 179-180: Should define "stable". This comparison, using data from Table 1, is NS by Chi-square.
Revised

5. Fig 2: Should include CIs for the risk estimates.
Figure 2 represents raw data of outcomes and no risk comparisons. Clarification on the suggestion would be appreciated. 95% CI of the prevalence of multiples or of risks between follicle numbers?

6. Fig 3: The chart could be misinterpreted by patients, since it does not include CIs for each of the estimates.

There is unfortunately no practice way for us to illustrate 95%CI for all the estimates and have the table remain simple and functional. A modifying statement to this effect was placed in the figure legend.

Page 23:
*Percentages are rounded to the closest whole number and represent mean outcomes from the study. 95%CI of the actual risk are not shown.

EDITORIAL OFFICE COMMENTS:

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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. Was this study presented at ASRM in 2018? If so, please disclose the name, date/s, and location of the meeting on the title page of your manuscript. Yes, this was presented at ASRM in 2018. This information has been updated on the title page.

4. 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.

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Titles should include "A Randomized Controlled Trial," "A Meta-Analysis," or "A Systematic Review," as appropriate, in a subtitle. Otherwise, do not specify the type of manuscript in the title.
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Original research category. Abstract Word count: 269

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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.

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