Date: Aug 24, 2018
To: "Lena Sagi-Dain" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-18-1379

RE: Manuscript Number ONG-18-1379

The yield of chromosomal microarray analysis among 5750 fetuses with various sonographic anomalies

Dear Dr. Sagi-Dain:

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 Sep 14, 2018, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1: Lena and colleagues present findings from a retrospective study designed to evaluate the role of chromosomal microarray analysis (CMA) in pregnancies with various ultrasound anomalies and to characterize the copy number variant patterns. The authors utilized data 5750 women who had the CMA performed in Israel from 2013-2017 (Israel Ministry of Health Database). They report that with various categories of fetal ultrasound abnormalities, CMA identified chromosomal abnormalities in 0.4-4.7% of cases. A point-by-point critique of the paper follows:

1) The authors note that they included all women who had an ultrasound abnormality noted and had CMA analysis performed. Presumably the CMA analysis was done on amniotic fluid following amniocentesis, however, this is never stated in the paper. How many women had an ultrasound abnormality and declines CMA analysis? If CMA was performed on amniotic fluid samples, where there any amniocentesis complications noted in the study cohort? Benefits of information from CMA should be weighed against risk for pregnancy loss or other post-amniocentesis complications. These additional specifics should be added to the revised paper.

2) Was there consistency among the 12 laboratories running the CMA analysis related to the CMA results? Was there any standardization of the CMA testing among the testing centers?

3) The authors report on Page 9 of the paper that one author categorized sonographic abnormalities and 2 other authors categorized CMA results into benign, pathogenic, likely pathogenic, karyotype detectable. Were these authors blinded to the CMA results for the former, and the ultrasound results for the latter? It would be important that these evaluators make their assessment in a blinded fashion to avoid any potential biases regarding assignment of categories. This should be clearly specified in the revised paper.

4) The authors report that all women with abnormal ultrasound imaging received genetic counseling for first line evaluation. This included women with hydramnios and IUGR. Women with soft markers only or abnormal first trimester screening results were excluded. What were the "soft markers" excluded? It would be helpful to provide this additional to the reader to better understand the study population.

5) Tables 1-3 are appropriate. Was the control population derived from the same population? This information would be important to specify in the revised paper.

6) Figure 1 is appropriate.

Reviewer #2: The purpose of this study was to examine the role of chromosomal microarray analysis in pregnancies with various sonographic anomalies and to characterize the copy number variants in diverse fetal phenotypes. The authors concluded that their data demonstrated the importance of performing microarray analysis in pregnancies with variable degrees of sonographic anomalies and non-structural sonographic abnormal findings. They argued that microarrays should not be limited only to severe anomalies.

The subject matter is proper for this Journal. The article structure is adequately set and easy to read and follow. All of the 22 references, 1 Figures and 4 tables are listed correctly.

Introduction:

1" Objectives were clear. What is the study hypothesis?

Methods:

2" The authors defined exclusion criteria. What is definition of mild pyelectasis and choroid plexus cyst?

3" Who performed ultrasound exams?

4" What was an indication for ultrasound exam?

Discussion:

5" This section of the manuscript is long and would benefit from consolidation.

6" The authors discussed study limitations. What are strengths of this study?

References:

7" References 2, 3, 5, 6-13,15, 16-22 are not quoted according to the Journal standards.

Reviewer #3: The submitted manuscript reviews a retrospective cohort of amniotic fluid samples drawn from gravidas with various sonographic abnormalities to evaluate their relationship with different categories of chromosomal microarray aberrations and comparing to standard karyotype. The manuscript is well written and well organized. The study methods have limitations but are proper. Much of the research findings are descriptive in nature, and the results are not particularly surprising. The study may contribute to the body of evidence supporting CMA to replace standard karyotype for evaluating chromosomal aberrations in pregnancies with ultrasound abnormalities.

Strengths:
Validity
Diagnostic uncertainty: All pregnancies from the study center undergoing CMA analysis for abnormal sonographic findings, excluding weak soft markers, reportedly were included in the analysis. Samples obtained for other reasons were excluded.

Comparisons: Direct comparisons were made between CMA and standard karyotype for each sonographic abnormality. CMA and karyotype testing were done on all amniotic fluid samples independently. Interpretation of all test results was independent and 100% objective.

Results
Strength of comparison: The study drew from a large cohort of subjects who had genetic amniocentesis. For all single abnormality groups, CMA uncovered significantly more aberrations than standard karyotype.

Practical application
Although several different laboratories were used, all appeared to be appropriately credentialed.

Weaknesses:
Validity
Diagnostic uncertainty: Demographic characteristics of the study and control subjects should be provided with more detail. All we know is that they came to Israel for their amniocentesis.

Comparisons: The control group did not come from the study population. Historical controls were used from other published studies.

Results
Postnatal follow-up for study subjects was lacking and is properly acknowledged in the Discussion section.

Practical application

Acceptance rate and completion rate of amniocentesis for potential subjects who demonstrated the qualifying sonographic abnormalities.

No information on whether or not test results lead to changes in management of the pregnancy were provided.

Variants of undetermined significance were more common in the study population than abnormal CMA results, overall and in all subgroups except cardiovascular abnormalities. These indeterminate results are potentially harmful in the stress and anxiety they provoke. This point was never discussed in the manuscript.

Questions
Do the authors believe the incremental benefit that CMA offers over standard karyotype mitigates the anxiety provoked by these indeterminate results to an acceptable level? If so, why?

Has the accuracy of CMA testing on amniotic fluid been determined relative to blood testing after birth?

STATISTICAL EDITOR'S COMMENTS:

1. lines 229-233: This section and Table 1 require further explanation. I assume that "compared to the remaining cohort" means that each columns numerator and denominator were used to compare to the overall column minus the entries for that column. If so, then the comparisons for polyhydramnios and for IUGR each have small entries of 3 and 1, thus requiring Fisher's test. When I try to replicate the stats, I obtain different answers, although still significant. Should have a better explanation for how these comparisons were done and verify the results.

2. lines 228-229: This comparison also needs better explanation. Ref (8) has 12362 patients, of whom 3090 had abnormal US, leaving a total of 9272, which is the same as that on line 229. However, in ref (8) the number of abnormal CNVs was 94 (lines 194-195). These data (272/5750 vs 94/9272) yield an OR = 4.85 [3.83-6.14], not 7.9. On the other hand, lines 197-199 seem to imply that the comparison group was ref(9), with 15225 US as the denominator. Need to clearly state what the comparison group was.

3. Table 1: The ratio of CMA to karyotype is not useful without CIs for the ratios and a statement as to the statistical meaning of any comparisons.

4. Table 2: Need to check the comparisons. the comparison of trisomy 21 multiple anomalies vs all others (assuming similar method as Table 1) does not yield $p < .0001$

5. Rather than fig 1. suggest a shortened version of Table 1 with karyotype and submicroscopic CNVs for each anomaly grouping, along with CIs.

6. Supple Table 1: need to include CIs for ratio of deletions/duplications and state any significance to differences.

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, as well as subsequent author queries. 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:
   1. OPT-IN: Yes, please publish my response letter and subsequent email correspondence related to author queries.
   2. OPT-OUT: No, please do not publish my response letter and subsequent email correspondence related to author queries.

2. 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.

3. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology will be transitioning as much as possible to use of the reVITALize definitions, and we encourage authors to familiarize themselves with them. The obstetric data definitions are available at http://links.lww.com/AOG/A515, and the gynecology data definitions are available at http://links.lww.com/AOG/A935.

4. 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 appendixes).

Please limit your Introduction to 250 words and your Discussion to 750 words.

5. Specific rules govern the use of acknowledgments in the journal. Please edit your acknowledgments or provide more information in accordance with 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 signature on the journal's author agreement 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).

6. 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.

7. 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.

8. 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.

9. Our readers are clinicians and a detailed review of the literature is not necessary. Please shorten the Discussion and focus on how your results affect or change actual patient care. Do not repeat the Results in the Discussion section.

10. 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.

11. The American College of Obstetricians and Gynecologists' (College) documents are frequently updated. These documents may be withdrawn and replaced with newer, revised versions. If you cite College 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. 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 a College document has been withdrawn, it should not be referenced in your manuscript (exceptions could include manuscripts that address items of historical interest). All College documents (eg, Committee Opinions and Practice Bulletins) may be found via the Resources and Publications page at http://www.acog.org/Resources-And-Publications.

12. The Journal's Production Editor had the following to say about the figures in your manuscript:

"Figure 1: Please upload a version of this figure with solid colored bars (colors are fine) and without a third dimension."

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.

Figures should be no smaller than the journal column size of 3 1/4 inches. Art that is low resolution, digitized, adapted from slides, or downloaded from the Internet may not reproduce. Refer to the journal printer's web site (http://cjs.cadmus.com/da/index.asp) for more direction on digital art preparation.

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If you choose to revise your manuscript, please submit your revision via Editorial Manager for Obstetrics & Gynecology at http://ong.editorialmanager.com. It is essential that your cover letter list point-by-point the changes made in response to each criticism. Also, please save and submit your manuscript in a word processing format such as Microsoft Word.

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

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 Sep 14, 2018, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

The Editors of Obstetrics & Gynecology

2017 IMPACT FACTOR: 4.982
2017 IMPACT FACTOR RANKING: 5th out of 82 ob/gyn journals

If you would like your personal information to be removed from the database, please contact the publication office.

To:
The Editor–in-Chief
Obstetrics and Gynecology
September 9th 2018

Dear Editors of Obstetrics & Gynecology,

We thank you for the opportunity to resubmit our manuscript entitled: "The yield of chromosomal microarray analysis among 5750 fetuses with various sonographic anomalies" for consideration for publication in Obstetrics and Gynecology.

We are very grateful to the reviewers and the editors for their thoughtful, supportive comments and suggestions. The manuscript has been substantially reformatted and modified based on these comments.
We have addressed the issue that was raised, and point-by-point responses to each comment are included in the cover letter, marked in red.

The manuscript is submitted solely to Obstetrics and Gynecology. It is not under consideration elsewhere, and will not be submitted elsewhere until a final decision is made by the editors of Obstetrics and Gynecology.

We affirm that this manuscript is an honest, accurate, and transparent account of the study being reported. All authors gave their agreement to the submission of the manuscript. No conflicts of interests are declared, and no funding was received.

In this manuscript we describe 5750 pregnancies in which CMA was performed due to various ultrasonographic fetal abnormalities, including structural anomalies, intrauterine growth restriction and polyhydramnios. The analysis of this large cohort reveals comprehensive data regarding the role of microscopic and submicroscopic chromosomal aberrations in fetuses with various sonographic phenotypes.
Thus, we hope you shall find this manuscript suitable for publication in Obstetrics and Gynecology.

Sincerely,
Lena Sagi-Dain, Shay Ben-Shachar.

Reviewer #1:

We wish to thank the reviewer for the in-depth analysis of our work and for raising several important points that needed clarification. We appreciate the time and effort expended on our behalf. We addressed each issue that was raised as follows:

1. The authors note that they included all women who had an ultrasound abnormality noted and had CMA analysis performed. Presumably the CMA analysis was done on amniotic fluid following amniocentesis, however, this is never stated in the paper. How many women had an ultrasound abnormality and declines CMA analysis? If CMA was performed on amniotic fluid samples, where there any amniocentesis complications noted in the study cohort? Benefits of information from CMA should be weighed against risk for pregnancy loss or other post-amniocentesis complications. These additional specifics should be added to the revised paper.

We thank the reviewer for this important comment. Unfortunately, the Israeli Ministry of Health database of prenatal CMA tests, used in our study for data acquisition, encompassed a limited number of parameters, including the indication for genetic testing, maternal age and gestational week of the invasive procedure, and the CMA result (the genomic coordinates and the interpretation of the individual laboratory). It did not include additional important information, such as the method and the complications of the invasive testing, or the count of women declining such testing.

The following was added to the "Limitations" section:

"Additional limitation is the lack of information regarding the acceptance rate and completion rates for amniocentesis both in the study and the control pregnancies, possibly affecting the generalizability of the results" (page 14, lines 317-320).

The following was added to the "Discussion" section:

Benefits of information from CMA should be weighed against risk for pregnancy loss or other post-amniocentesis complications. However, the risk related to amniocentesis was recently shown to be lower than previously thought, about 1:800, enhancing the advantage of the potential benefits of CMA over the risk of pregnancy loss associated with invasive prenatal procedures (1) (pages 16-17, lines 372-376).

2. Was there consistency among the 12 laboratories running the CMA analysis related to the CMA results? Was there any standardization of the CMA testing among the testing centers?

We agree that consistency is essential when comparing different labs and therefore made an effort to provide high consistency of the results. CMA analyses were performed using either SNP-based array or comparative genomic hybridization. All tests were provided by clinical laboratories approved by the Israeli Ministry of Health. To promote consistency in interpretation and reporting of genomic microarray results, all standards and reports were based on the recommendation of the Israeli Medical Genetic association. These recommendations were based on the guidelines provided by the American College of Medical Genetic and Genomics (2, 3). In addition, to further increase consistency each finding was reevaluated by the study team.
This was mentioned in the Methods section (Page 8, lines 158-161).

3. The authors report on Page 9 of the paper that one author categorized sonographic abnormalities and 2 other authors categorized CMA results into benign, pathogenic, likely pathogenic, karyotype detectable. Were these authors blinded to the CMA

results for the former, and the ultrasound results for the latter? It would be important that these evaluators make their assessment in a blinded fashion to avoid any potential biases regarding assignment of categories. This should be clearly specified in the revised paper.
Dr. Shay Ben Shachar was blinded to the sonographic indication while going over the CMA results.
Dr. Lena Sagi-Dain has categorized the 5750 sonographic anomalies into several pre-defined subgroups (e.g. brain anomalies, polyhydramnios, etc.), and thus was not blinded to the CMA results.
The following was added to the "Methods" section:
"The CMA findings were classified by the clinical laboratories according to the accepted clinical guidelines (4) and reviewed by two authors (SDL and SBS) and categorized into normal (including benign and VOUS; likely benign categories), pathogenic, likely pathogenic, VOUS or "karyotype-detectable" (i.e., CNVs at least 10 Mb in size)). The latter author was blinded to the sonographic indication" (Page 9, lines 184-188).

4. The authors report that all women with abnormal ultrasound imaging received genetic counseling for first line evaluation. This included women with hydramnios and IUGR. Women with soft markers only or abnormal first trimester screening results were excluded. What were the "soft markers" excluded? It would be helpful to provide this additional to the reader to better understand the study population.
The definition of soft markers according to the Israeli position paper (2013) was added as Supplementary Table 1 (page 6, lines 105-106).

The soft marker	**Definition**
Short femur	Femoral length less that two standard deviations below the mean
Echogenic cardiac focus	Intracardiac white point demonstrated at four-ventricle view, with echogenicity similar to that of bony structures
Choroid plexus cyst	A cystic finding below 3 mm in the choroid plexus of the lateral ventricles of the fetal brain
Mild pyelectasis	≥4 mm at gestational age of 14+0-19+6 weeks ≥6 mm at gestational age of 20+0-29+6 weeks ≥7 mm at gestational age of ≥30+0 weeks The finding of renal pelvis dilated to above 10 mm is defined as hydronephrosis and mandates a referral to genetic counseling.
Single umbilical artery	Two vessels in the umbilical cord

5. Tables 1-3 are appropriate. Was the control population derived from the same population? This information would be important to specify in the revised paper.
This is a crucial point, and we thank the reviewer for emphasizing it.
Two different control populations were used in the study. The overall frequency of abnormal CMA findings in our cohort was compared to a control population of 9272 pregnancies with normal ultrasound findings described in a systematic review by Callaway et al., encompassing 5108 tests due to advanced maternal age, and 4164 due to "other ascertainment causes", including fetuses with abnormal Down screening tests, family history, previous pregnancy with chromosome abnormality and parental

request (5). This group yielded 94 (1.03%) CNVs with associated clinical significance.
This control group was not related to our population.
The frequency of 6 most frequent abnormal CMA findings in our cohort was compared to another control population, encompassing 15,225 Israeli pregnancies with normal ultrasound findings (6). This cohort is derived from the comparable population to the study group.
The matter was emphasized in the article (pages 9-10, lines 193-202).

6. Figure 1 is appropriate .

Reviewer #2:

We are grateful to the reviewer for providing us with a valuable critique and for pointing out several elements that needed clarification. The changes and additions that we made using the reviewer's comments as guidelines are as follows:

Introduction:

1. Objectives were clear. What is the study hypothesis ?

The study hypothesis was that CMA testing has an important value in pregnancies with various sonographic anomalies, and that the results depend on the specific sonographic phenotype.

Methods :

2. The authors defined exclusion criteria. What is definition of mild pyelectasis and choroid plexus cyst?

The definition of soft markers according to the Israeli position paper (2013) was added as Supplementary Table 1 (page 6, lines 105-106).

The soft marker	**Definition**
Short femur	Femoral length less that two standard deviations below the mean
Echogenic cardiac focus	Intracardiac white point demonstrated at four-ventricle view, with echogenicity similar to that of bony structures
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Single umbilical artery	Two vessels in the umbilical cord

3. Who performed ultrasound exams?

4. What was an indication for ultrasound exam?

(The answer relates to both questions 3 and 4)

The imaging findings were detected by a first or second trimester sonographic anatomic survey, which is routinely performed in the majority of pregnant Israeli women by qualified sonographers. Polyhydramnios and IUGR could also be demonstrated at routine second and/or third trimester sonographic evaluation, which normally includes fetal weight estimation and amniotic fluid measurement (added to page 6, lines 107-111).

Discussion :

5. This section of the manuscript is long and would benefit from consolidation.

The Discussion section was shortened significantly.

6. The authors discussed study limitations. What are strengths of this study?

The main value of our study is in describing a large number of prenatal CMA tests, with subdivision by sonographic groups and frequencies of common CNVs (added to page 15, lines 328-329).

References:

7. References 2, 3, 5, 6-13,15, 16-22 are not quoted according to the Journal standards.
The referenced were corrected in accordance with the Journal standards.

Reviewer #3:

We thank the reviewer for the time and effort expended on our behalf to enhance the presentation of our investigation. We appreciate the valuable comments and made the following additions and changes accordingly.

Strengths: Validity
Diagnostic uncertainty: All pregnancies from the study center undergoing CMA analysis for abnormal sonographic findings, excluding weak soft markers, reportedly were included in the analysis. Samples obtained for other reasons were excluded.

Comparisons: Direct comparisons were made between CMA and standard karyotype for each sonographic abnormality. CMA and karyotype testing were done on all amniotic fluid samples independently. Interpretation of all test results was independent and 100% objective.

Results
Strength of comparison: The study drew from a large cohort of subjects who had genetic amniocentesis. For all single abnormality groups, CMA uncovered significantly more aberrations than standard karyotype.

Practical application
Although several different laboratories were used, all appeared to be appropriately credentialed.

Weaknesses: Validity
Diagnostic uncertainty: Demographic characteristics of the study and control subjects should be provided with more detail. All we know is that they came to Israel for their amniocentesis.

The Israeli Ministry of Health database of prenatal CMA tests, used in our study for data acquisition, encompassed a limited number of parameters, including the indication for genetic testing, maternal age and gestational week of the invasive procedure, and the CMA result (the genomic coordinates and the interpretation of the individual laboratory).
Thus, unfortunately, we did not have an access to any other important information.
This was added to the "limitations" section (page 14, lines 320-322).

Comparisons: The control group did not come from the study population. Historical controls were used from other published studies .

We agree with this comment. The following was added to the "limitations" section: "Furthermore, the study and the control groups might not be comparable in terms of several important confounders, such as maternal age (not noted in the control groups), the use of NIPT or the results of biochemical screening for Down syndrome" (page 14, lines 320-322).

Results
Postnatal follow-up for study subjects was lacking and is properly acknowledged in the Discussion section.

Practical application

Acceptance rate and completion rate of amniocentesis for potential subjects who demonstrated the qualifying sonographic abnormalities.
No information on whether or not test results lead to changes in management of the pregnancy were provided.
Unfortunately, the Israeli Ministry of Health database did not include additional important information, such as the acceptance and completion rates of amniocentesis (page 14, lines 317-320), or whether the test results lead to changes in management of the pregnancy.

Variants of undetermined significance were more common in the study population than abnormal CMA results, overall and in all subgroups except cardiovascular abnormalities. These indeterminate results are potentially harmful in the stress and anxiety they provoke. This point was never discussed in the manuscript.
The study provides a large-scale data regarding the detection rate of pathogenic copy number variations and variant of unknown significance detected by CMA in different fetal structural anomalies. VOUS are a common "by-product" not only in CMA testing but in next generation sequencing as well. The increased number of VOUS warranties detailed explanation prior to the procedure and generation of policy intended to decrease anxiety, while providing the essential information regarding bone fide chromosomal aberrations leading to severe diseases. In Israel, for example, the routine genetic counseling prior to invasive prenatal testing includes a detailed explanation about VOUS findings, including the possible parental anxiety. Each couple is given an option not to be informed about VOUS findings. Thus, the parents decide whether they are interested in receiving all the information from CMA testing, or in skipping findings of uncertain significance. We believe this protocol is helpful in mitigating the possible anxiety. This point is now discussed in the manuscript (pages 14-15, lines 323-326).

Questions
Do the authors believe the incremental benefit that CMA offers over standard karyotype mitigates the anxiety provoked by these indeterminate results to an acceptable level? If so, why?
We agree with the reviewer. Please see our response above.

Has the accuracy of CMA testing on amniotic fluid been determined relative to blood testing after birth?
Verifying prenatal CMA testing result by postnatal blood testing is not an accepted protocol in Israel, as amniotic CMA is based on DNA testing and considered accurate. Nevertheless, we did not have access to any postnatal data.
This was noted in the "limitations" section (page 14, lines 313-314).

Statistical editor's comments:

We are grateful to the reviewer for the valuable in-depth analysis of our paper and for the important comments and suggestions for enhancing it. We addressed each of them as follows:

1. Lines 229-233: This section and Table 1 require further explanation. I assume that "compared to the remaining cohort" means that each columns numerator and denominator were used to compare to the overall column minus the entries for that column. If so, then the comparisons for polyhydramnios and for IUGR each have small entries of 3 and 1, thus requiring Fisher's test. When I try to replicate the stats, I obtain different answers, although still significant. Should have a better explanation for how these comparisons were done and verify the results.
Following reviewer's righteous comment, all calculations were re-calculated using Fisher's exact test. P-values were corrected (whereas the statistical significance remained as in the original manuscript) (page 20).

2. Lines 228-229: This comparison also needs better explanation. Ref (8) has 12362 patients, of whom 3090 had abnormal US, leaving a total of 9272, which is the same as that on line 229. However, in ref (8) the number of abnormal CNVs was 94 (lines 194-195). These data (272/5750 vs 94/9272) yield an OR = 4.85 [3.83-6.14], not 7.9. On the other hand, lines 197-199 seem to imply that the comparison group was ref(9), with 15225 US as the denominator. Need to clearly state what the comparison group was.
We thank the editor for this comment. Two different control populations were used in the study.
1. The overall frequency of abnormal CMA findings in our cohort was compared to a control population of 9272 pregnancies with normal ultrasound findings described in a systematic review by Callaway et al., encompassing 5108 tests due to advanced maternal age, and 4164 due to "other ascertainment causes", including fetuses with abnormal Down screening tests, family history, previous pregnancy with chromosome abnormality and parental request (5). This group yielded 94 (1.03%) CNVs with associated clinical significance.
2. The frequency of 6 most frequent abnormal CMA findings in our cohort was compared to another control population, encompassing 15,225 Israeli pregnancies with normal ultrasound findings (6).
The matter was emphasized in the article (pages 9-10, lines 193-202).
The OR was corrected, and we thank the reviewer for noticing this miscalculation.

3. Table 1: The ratio of CMA to karyotype is not useful without CIs for the ratios and a statement as to the statistical meaning of any comparisons.
Confidence intervals of proportions were calculated using modified Wald method (added to Methods section – page 10, lines 215-216, and Tables 1 and 2 – pages 20-21 and 22-23, respectively).

4. Table 2: Need to check the comparisons. The comparison of trisomy 21 multiple anomalies vs all others (assuming similar method as Table 1) does not yield $p < .0001$
The comparisons were re-checked, and p-values were corrected (Table 2, page 22-23).

5. Rather than fig 1. suggest a shortened version of Table 1 with karyotype and submicroscopic CNVs for each anomaly grouping, along with CIs.
We thank the editor for this suggestion. Figure 1 was omitted accordingly.

6. Supple Table 1: need to include CIs for ratio of deletions/duplications and state any significance to differences.
Confidence intervals of proportions were added (now Supplementary Table 2, page 26).

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, as well as subsequent author queries. 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:
1. OPT-IN: Yes, please publish my response letter and subsequent email correspondence related to author queries.
2. OPT-OUT: No, please do not publish my response letter and subsequent email correspondence related to author queries.
Yes, please publish our response letter and subsequent email correspondence related to author queries.

2. 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.
The data was manually entered over the specified time period (1/2013 and 09/2017) by the head of community genetics department in the Israeli Ministry of Health. We had no means to verify this information.

3. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology will be transitioning as much as possible to use of the reVITALize definitions, and we encourage authors to familiarize themselves with them. The obstetric data definitions are available at http://links.lww.com/AOG/A515, and the gynecology data definitions are available at http://links.lww.com/AOG/A935.
We went over the reVITALize definitions.

4. 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 appendixes).
Please limit your Introduction to 250 words and your Discussion to 750 words.
Introduction and Discussion sections were shortened significantly. The Introduction now encompasses 239 words, and the Discussion – from initial 1082 to 892 words (as we had to add numerous comments in response to reviewers' questions).
If the Editors find this unacceptable, we will be happy to try and cut down more words.

5. Specific rules govern the use of acknowledgments in the journal. Please edit your acknowledgments or provide more information in accordance with 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 signature on the journal's author agreement 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).
Guidelines related to acknowledgments were reviewed and followed.

6. 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 .
The abstract was reviewed. Its length is 192 words.

7. 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 .

8. 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.
The symbol was not used.

9. Our readers are clinicians and a detailed review of the literature is not necessary. Please shorten the Discussion and focus on how your results affect or change actual patient care. Do not repeat the Results in the Discussion section.
The discussion was shortened.

10. 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.
The tables were adjusted to journal style.

11. The American College of Obstetricians and Gynecologists' (College) documents are frequently updated. These documents may be withdrawn and replaced with newer, revised versions. If you cite College 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. 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 a College document has been withdrawn, it should not be referenced in your manuscript (exceptions could include manuscripts that address items of historical interest). All College documents (eg, Committee Opinions and Practice Bulletins) may be found via the Resources and Publications page at http://www.acog.org/Resources-And-Publications.
Reference list was reviewed, appropriately.

12. The Journal's Production Editor had the following to say about the figures in your manuscript.
"Figure 1: Please upload a version of this figure with solid colored bars (colors are fine) and without a third dimension".
Based on the suggestion of the statistical editor, Figure 1 was omitted.

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 .
Figures should be no smaller than the journal column size of 3 1/4 inches. Art that is low resolution, digitized, adapted from slides, or downloaded from the Internet may not reproduce. Refer to the journal printer's web site (http://cjs.cadmus.com/da/index.asp) for more direction on digital art preparation.

References:

1. Wulff CB, Gerds TA, Rode L, Ekelund CK, Petersen OB, Tabor A. Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147,987 singleton pregnancies. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2016;47(1):38-44.
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6. Maya I, Sharony R, Yacobson S, Kahana S, Yeshaya J, Tenne T, et al. When genotype is not predictive of phenotype: implications for genetic counseling based on 21,594 chromosomal microarray analysis examinations. Genetics in medicine : official journal of the American College of Medical Genetics. 2017.

Daniel Mosier

From: [redacted]
Sent: Thursday, September 13, 2018 3:48 PM
To: Daniel Mosier
Subject: Re: Manuscript Revisions: ONG-18-1379R1
Attachments: Editorial comments.doc; 18-1379R1 ms revised clean.doc; 18-1379R1 ms revised.doc

Dear Editors,

We thank you for the opportunity to resubmit our manuscript entitled: "Chromosomal microarray analysis results from pregnancies with various ultrasonographic anomalies" for consideration for publication in Obstetrics and Gynecology.

We are grateful for the in-depth analysis of our work and for raising several important points that needed clarification. We appreciate the time and effort expended on our behalf.
As suggested by the Editors, Supplementary Tables were included in the main body.

We addressed each issue that was raised as in the file named "Editorial comments". In addition, attached are the revised and the clean versions of the manuscript.

On behalf of all authors,
Lena Sagi-Dain, Shay Ben-Shachar.

On Tue, Sep 11, 2018 at 6:42 PM Daniel Mosier <dmosier@greenjournal.org> wrote:

> Dear Dr. Sagi-Dain,
>
> Thank you for submitting your revised manuscript. It has been reviewed by the editor, and there are a few issues that must be addressed before we can consider your manuscript further:
>
> 1. Please note the minor edits and deletions throughout. Please let us know if you disagree with any of these changes.
> 2. The editing process may have disturbed the order of your references. When you review your manuscript, please check that the references and references and citations are appropriately number and cited throughout your paper.
> 3. LINE 5: Please list the authors' names in this format: first name, last name, academic degrees.
> 4. LINE 6: Please the following authors to respond to his/her authorship confirmation email. We emailed him/her at the email addresses listed with each name below. The email contains a link that needs to be clicked on. The sender of the email is EM@greenjournal.org.
>
> - Reeval Segel: [redacted]
> - Esther Manor: [redacted]
> - Amihood Singer: [redacted]
>
> 5. LINE 55: Please make clear what tissue or fluid was evaluated-Are these all amniocenteses?

6. LINE 57: Please change to fetal growth restriction (FGR) everywhere
7. LINE 63: Where are these data stated in the body of your paper? If the data are not contained in the text or tables, please add them
8. LINE 67: You do not mention these in your methods-where did they come from
9. LINE 99:
    a. From where?
    b. Again -On what fluid or tissue?
10. LINE 105: Please revise "and/or" to mean either "and" or "or." Be sure this is done throughout your paper.
11. LINE 230: Lower than what-seems higher than the control cohort
12. LINE 237:

    a. I don't understand the comparisons in this paragraph-why not just present the various rates
    b. It would seem better to not make statistical comparisons to the overall cohort but rather present rates and their 95% confidence intervals
13. TABLE 1: Cite with a superscript here
14. TABLE 5: Please express this p-value and all the p-values in your paper to no more than three decimal places.

Each of these points are marked in the attached manuscript. Please respond point-by-point to these queries in a return email, and make the requested changes to the manuscript. When revising, please leave the track changes on, and do not use the “Accept all Changes” function in Microsoft Word.

Please let me know if you have any questions. Your prompt response to these queries will be appreciated; please respond no later than COB on **Thursday, September 13th.**

Sincerely,

-Daniel Mosier

Daniel Mosier

Editorial Assistant

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