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 03, 2021
To: "Anne Mette Skov Sørensen" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-21-2085

RE: Manuscript Number ONG-21-2085

Exposure to tramadol during early pregnancy: A Danish nationwide cohort study

Dear Dr. Sørensen:

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

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

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

Your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Dec 24, 2021, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1:

This is a propensity score matched cohort study of tramadol exposure in early pregnancy with the outcomes of spontaneous abortion and congenital anomalies. The use of propensity scores is well described and helpful for analysis of medication exposure. I appreciate this study's contribution to understanding of tramadol exposure risk.

1. Precis and Abstract conclusion: This is a strong study - consider strengthening your language from "does not appear" to "is not" or similar.
2. Introduction: Please justify the need for additional studies (yours). Specifically name risk factors for tramadol use that may also increase risk of spontaneous abortion or congenital anomaly. Consider explaining that propensity score analysis aims to account for this but requires a large database, such as the one you have.
3. Materials and methods, line 117: Why do you exclude stillbirths? I see in your sensitivity analyses you have one focusing on terminations of pregnancy after 12 weeks, presumably to get at termination due to congenital anomaly. There is an increased risk of stillbirth with many congenital anomalies. This study would be strengthened by including stillbirths and, if possible, noting ratio of stillbirths with anomalies.
4. Analyses: Did you consider including age as a variable when building propensity scores? Increased maternal age may increase likelihood of painful conditions needing treatment and it certainly increases spontaneous abortion and congenital anomaly.
5. Outcome analysis: Can you explain why you chose a Cox proportional hazards regression model for analysis of spontaneous abortion? You do not include a time factor, so this is surprising to me.
6. Discussion, line 247: please include the incidence of neonatal abstinence syndrome with tramadol exposure.
7. Discussion, lines 249-253: Can you comment on who is missed by these registries? Those who experience spontaneous abortion at home without any hospital or office encounter would be missed. I do not believe these people would change your findings, but I think it is important to admit.

Reviewer #2:

This is a much needed study: the effects of many medications commonly prescribed to pregnant women are still poorly characterized. The authors undertook an important task: to characterize the structural effects on the developing fetus of a commonly used opioid, tramadol.
One concern I have is that most of the women in the study are on polytherapy with several other medications. and, while my concern is alleviated by the fact that women on tramadol tend to be on a similar or higher number of medications, we know that even within the same class of medications outcomes can vary greatly with different medications. However, given careful matching/selection of their cohorts, a stochastic distribution is likely. I would like the authors to report also the % MCM in those 653 pregnancies with exposure to tramadol monotherapy. That is a high enough number to provide valuable information, less tainted by other exposures. If feasible, an analysis of dose-dependent effect in this monotherapy group would also be valuable
Another important observation is that the rate of major congenital malformations is higher than what is reported in the literature (1-3%) and closer to 4%. That is itself is a concern and, while the control cohort has a similar rate of MCMs, it is matched for the study cohort for comorbidities/medications. This may arguably suggest that perhaps tramadol exposure may be comparable with other medications in terms of risk of MCMs, given such high proportion of women on polytherapy in both of their cohorts, but that risk does seem higher than the risk published in the general population.
Most importantly, the authors should correct their conclusion in the body text: "we believe that use of tramadol during early pregnancy does not pose a risk for the foetus". I like better their conclusion in the abstract. They are not looking at all the risks an exposure can bring for the foetus, they are strictly looking at MCM and abortions. The exposure, even in the first trimester only, may be important for other neonatal outcomes (e.g. birth weight) and neurodevelopment.
Of note, there is a redundancy in your introduction - Line 71-72 : "exposure to analgesics before a pregnancy is acknowledged is common"; "is acknowledged" should be removed.
This is overall an excellent study.
-

Reviewer #3:

This study investigates the associations between early pregnancy exposure to tramadol, the most common prescription opioid in Denmark, and two outcomes, i.e., spontaneous pregnancy loss and congenital malformations, using data from the Danish National Registries (1997-2016). Propensity score matching was used to control for confounding. The investigators concluded that no associations were observed, although they admit that they cannot rule out the possibility of confounding by factors not available in the registry data, such as alcohol use.

There are strengths of the study, including its size and relatively complete data. The findings are assuring, given the difficulty for clinicians and patients to treat maternal pain symptoms while also safeguarding the health of the fetus. Still, I have several questions, most of which are clarifying the methods employed as well as disclosure of possible biases.

1. The paper describes the database as including "abortive outcomes with a hospital contact" (page 5 line 101). Does this mean that there may be spontaneous abortions excluded from the cohort if they did not involve a hospital visit? I understand why losses before 6 weeks were not included in the database. Still, I have some concern that the data may be affected by left truncation due to exclusion of some losses, as they may not be captured in the database. This is a potentially important limitation of the spontaneous loss findings, as left truncation, particularly if differential with respect to exposure status, can bias findings (Schisterman 2013).

2. Please clarify why non-livebirths were excluded from congenital malformation analysis. Is it possible that this exclusion could have resulted in selection bias for certain subgroups of defects, as pregnancies may be terminated upon recognition of the malformation during obstetric ultrasound and some defects carry high risk of mortality (Heinke 2020a, Heinke 2020b)?

3. The definition of the exposure classification and the possibility of misclassification warrant further discussion. The exposures were based on prescription data. Was the exposure period of interest (i.e., start of pregnancy through 12 weeks for malformations and start of pregnancy through 22 weeks for early losses) defined based on the prescription/fill date only, or based on the estimated period of use (based on number and frequency of doses covered by the prescription)? Also, did the prescriptions include both continuous and sporadic periods of use? If sporadic, and if the data are available, why wasn't the exposure analyzed at the day or week level? This seems particularly relevant for congenital malformations as the periods of organogenesis are very specific. Exposure as late as 11 or 12 weeks of gestation may be after onset of some malformations. The timing issue may also be relevant for studying the effect of tramadol on pregnancy loss, if the mechanism results from an acute exposure. Since Cox proportional hazard models were used for the pregnancy loss analysis, it is unclear why time-varying exposure was not taken into account to evaluate this timing issue. Lastly, how were prescription/use of other types of opioids taken into account? Did you consider excluding women with prescriptions for other types of opioids or diagnosis of opioid use disorder to have a cleaner comparison? I raise these concerns because, while not always the case, non-differential misclassification can result in a bias towards the null, which may credibly explain the null findings without a more quantitative assessment of this form of bias.

Minor Comments:
4. The paper lacks discussion of prior research on opioid use and risk of spontaneous abortions (e.g., Flannagan 2020). Is there reason to hypothesize the mechanism specific to tramadol would be different from other types of opioids?

5. How were correlations between multiples (e.g., twins) and between successive pregnancies of the same person over time handled in the analysis?

6. Since variable ratio matching was applied, were the matching strata (e.g., 1:1 versus 1:3) taken into account in the analysis?

7. Page 6 lines 119-120 "Gestational age was generally calculated from the first day of the last menstrual period and subsequently confirmed by ultrasonography." For instances where this was not the case, how else was gestational age assigned?

8. In the Methods, clarify what was done with medically induced abortions in the main models analyzing spontaneous losses.

9. What was the time scale used in the Cox models?

10. How were the malformation subgroupings defined (i.e., which defects comprised each subgroup)?

11. Page 9 lines 205-206. "None of the risk estimates for subgroupings of major congenital malformations reached statistical significance" I recommend describing the effect estimates rather than solely relying on statistical significance. To quote the Journal's author guidelines "Putting the results in the form of an effect size makes the result of the statistical test more clinically relevant and gives better context" In particular, it appears that there may be something occurring with nervous system defects (RR 1.8, 95% CI 1.0, 3.6; Figure 3).

12. In the Discussion the investigators noted "This study has several strengths including use of the Danish nationwide registries allowing for a detailed characterization of the population with minimal loss to follow-up." Were any participants excluded due to loss to follow-up? I don't recall seeing this noted in the eligibility flowchart.

13. Page 11 line 252 If the congenital defects have a positive predictive value of 89%, as noted, wouldn't this imply that ~10% of infants classified as having a defect don't actually have a defect? Could this outcome misclassification contribute to a bias towards the null? Is outcome classification better for certain defect subgroups?

14. The following text is unclear: Page 4 lines 85-86 "Few studies have specifically assessed the risk of tramadol use in early pregnancy and these results have neither been conclusive." Is this meant to read "have been inconclusive"?

15. Possible typos: Page 4 line 76 "200.000 adult users" should be "200,000 adult users"; Page 5 line 105 missing "materials" at the end of the following line "A description of the registers is provided in the supplementary."; Page 8 lines 189-190 "7,310 pregnancies were exposed to tramadol at some time from conception and 22 weeks onwards" should instead end something like "from conception through 22 weeks"; Page 9 lines 200-201 "The majority (n=3,630) of the pregnancies was singleton pregnancies." should be "were singleton"

References:
Heinke D, Nestoridi E, Hernandez-Diaz S, Williams PL, Rich-Edwards JW, Lin AE, et al. Risk of stillbirth for fetuses with specific birth defects. Obstet Gynecol. 2020a;135(1):133-40. PMID: 31809437.

Heinke D, Rich-Edwards JW, Williams PL, Hernandez-Diaz S, Fisher SC, Desrosiers TA, et al. Quantification of selection bias in studies of risk factors for birth defects among livebirths. Paediatric and Perinatal Epidemiology. 2020b;34(6):655-64. PMID: 32249969.

Flannagan KS, Mumford SL, Siaarda LA, Radoc JG, Perkins NJ, Andriessen VC, Zolton JR, Silver RM, Schisterman EF. Is opioid use safe in women trying to conceive? Epidemiology. 2020;31(6):844-51. PMID: 33311959

Schisterman EF, Cole SR, Ye A, Plagtt RW. Accuracy loss due to selection bias in cohort studies with left truncation. Paediatric and Perinatal Epidemiology. 2013;27(5):491-502. PMID: 23930785.

STATISTICS EDITOR COMMENTS:

Lines 40-46, Fig 2: While it is true that the rates of abortion and rates of major congenital malformations were similar, the

studies were underpowered to to discern the differences found. For instance, for abortions, the HR = 1.06, with abortion rates of 12.2 in unexposed and 11.9 % in unexposed pregnancies. Using alpha = 0.05, power = 0.80 and the sample sizes at hand, the minimum detectable HR would be ~ 1.10. So, one can only conclude that the difference is not likely to exceed ~ 10%. For the analysis of major congenital malformations, the math is worse, since the samples and the rates of adverse events are both smaller. Applying the same sample size/ power criteria, the minimum detectable RR is ~ 1.25. For the individual subgroups of malformations, the math is much worse, again owing to the lower rates. Should simply report the rates of adverse outcomes along with relevant CIs, but cannot generalize that there is not a statistical difference, based on these data.

EDITOR COMMENTS:

Please more explicitly speak to statistical power limitations, especially for the sub-group analyses.

EDITORIAL OFFICE COMMENTS:

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

A. OPT-IN: Yes, please publish my point-by-point response letter.
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* Include clinical trial registration numbers, PROSPERO registration numbers, or URLs at the end of the abstract (if applicable).
* Name the IRB or Ethics Committee institution in the Methods section (if applicable).
* Add any information about the specific location of the study (ie, city, state, or country), if necessary for context.

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4. If your study uses ICD-10 data, please make sure you do the following:

a. State which ICD-10-CM/PCS codes or algorithms were used as Supplemental Digital Content.
b. Use both the diagnosis and procedure codes.
c. Verify the selected codes apply for all years of the study.
d. Conduct sensitivity analyses using definitions based on alternative codes.
e. For studies incorporating both ICD-9 and ICD-10-CM/PCS codes, the Discussion section should acknowledge there may be disruptions in observed rates related to the coding transition and that coding errors could contribute to limitations of the study. The limitations section should include the implications of using data not created or collected to answer a specific research question, including possible unmeasured confounding, misclassification bias, missing data, and changing participant eligibility over time.
f. The journal does not require that the title include the name of the database, geographic region or dates, or use of database linkage, but this data should be included in the abstract.
g. Include RECORD items 6.3 and 7.1, which relate to transparency about which codes, validation method, and linkage were used to identify participants and variables collected.

5. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

6. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Original Research reports should not exceed 5,500 words; Case Reports should not exceed 2,000 words. Stated word limits include the title page, précis, abstract, text, tables, boxes, and figure legends, but exclude references.

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).
* If your manuscript was uploaded to a preprint server prior to submitting your manuscript to Obstetrics & Gynecology, add the following statement to your title page: "Before submission to Obstetrics & Gynecology, this article was posted to a preprint server at: [URL]."

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 limit for Original Research articles is 300 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.

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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.

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13. Line 224: Your manuscript contains a priority claim. We discourage claims of first reports since they are often difficult to prove. How do you know this is the first report? If this is based on a systematic search of the literature, that search should be described in the text (search engine, search terms, date range of search, and languages encompassed by the search). If it is not based on a systematic search but only on your level of awareness, it is not a claim we permit.

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* A confirmation that you have read the Instructions for Authors (http://edmgr.ovid.com/ong/accounts/authors.pdf), and
* A point-by-point response to each of the received comments in this letter. Do not omit your responses to the Editorial Office or Editors' comments.

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

Again, your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Dec 24, 2021, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Dwight J. Rouse, MD
Editor-in-Chief

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

Cover letter, revision

Dear editor-in-chief Dwight J. Rouse,

Thank you very much for your consideration of our revised manuscript. I, as corresponding author, confirm that I have read the Instructions for Authors. We are grateful for the constructive and valuable criticism and suggestions from reviewers and editors. Below is a point-by-point response to each of the received comments including responses to the Editorial Office and Editors' comments. Our responses and changes to the revised manuscript are written in red. Page and line numbers refer to the revised manuscript without track-changes.

We hope the revised manuscript is now suitable for publication.

Sincerely,
Anne Mette Skov Sørensen and co-authors

Reviewer comments. Manuscript Number ONG-21-2085
Exposure to tramadol during early pregnancy: A Danish nationwide cohort study

Reviewer #1:
This is a propensity score matched cohort study of tramadol exposure in early pregnancy with the outcomes of spontaneous abortion and congenital anomalies. The use of propensity scores is well described and helpful for analysis of medication exposure. I appreciate this study's contribution to understanding of tramadol exposure risk.
Reviewer comment 1) Precis and Abstract conclusion: This is a strong study - consider strengthening your language from "does not appear" to "is not" or similar.
Authors reply 1) We consider our study to be strong, among other, due to the large cohort. However, we prefer the more careful formulation with 'does not appear' considering the width of the confidence intervals and lack of power in the subgroup analyses.

Reviewer comment 2) Introduction: Please justify the need for additional studies (yours). Specifically name risk factors for tramadol use that may also increase risk of spontaneous abortion or congenital anomaly. Consider explaining that propensity score analysis aims to account for this but requires a large database, such as the one you have.

Authors reply 2) Thank you for this relevant addition to the introduction. We consider our study to be relevant as use of tramadol is high, pain is prevalent among women, and the current knowledge is limited. We have stated this in the introduction, and added the following on Page 4, Line 103: 'Due to the widespread use of tramadol, and the lack of conclusive results, increased knowledge on potential risks associated with drug exposure during pregnancy is deemed relevant. The aim of the study is to assess the risk of congenital malformations and spontaneous abortions following exposure to tramadol in early pregnancy by conducting propensity score matched analyses accounting for a broad range of risk factors'.

Reviewer comment 3) Materials and methods, line 117: Why do you exclude stillbirths? I see in your sensitivity analyses you have one focusing on terminations of pregnancy after 12 weeks, presumably to get at termination due to congenital anomaly. There is an increased risk of stillbirth with many congenital anomalies. This study would be strengthened by including stillbirths and, if possible, noting ratio of stillbirths with anomalies.

Authors reply 3) Thank you for this relevant comment and good suggestion. It is correct, that we conducted the sensitivity analysis on pregnancy terminations after 12 weeks to estimate terminations due to congenital malformations. These late terminations were approved in cases with a malformation. We chose only to include the following adverse events: spontaneous abortions, major congenital malformations, and elective terminations due to the sample size. However, we do acknowledge that other adverse events, such as stillbirths, could have been included and would have been interesting to investigate. In Denmark, the incidence rates of stillbirths are stable around 0.3% per year, so based on a prespecified assumption of a small sample size, we chose not to include this outcome as such an analysis would be limited with regards to power.

Reviewer comment 4) Analyses: Did you consider including age as a variable when building propensity scores? Increased maternal age may increase likelihood of painful conditions needing treatment and it certainly increases spontaneous abortion and congenital anomaly.

Authors reply 4) We agree that age is a relevant covariate in analyses of spontaneous abortions and congenital malformations, hence we included age in the propensity score model based on, among other,

the arguments put forth by the reviewer. Age was included as separate age groups (≤19, 20-24, 25-29, 30-34, and ≥35). We have highlighted this in the method section on Page 6, Line 150: 'The propensity scores were estimated using a logistic regression model and were based on predefined variables including age, socioeconomic status, previous pregnancy history (parity and previous event of interest), smoking, prescription drug use as well as health care use in the year prior to the date of conception (supplementary Table 2)'.

Reviewer comment 5) Outcome analysis: Can you explain why you chose a Cox proportional hazards regression model for analysis of spontaneous abortion? You do not include a time factor, so this is surprising to me.

Authors reply 5) We chose the Cox proportional hazards regression model as we did account for the time factor. Pregnant women only contributed to the risk time while being pregnant between the index date (exposure date) and gestational age week 22. Thus, pregnancies that were terminated (e.g. induced abortions) prior to week 22 were censored. We agree on the ambiguity, and have rephrased the section on Page 7, line 167: 'We used a Cox proportional hazards regression model to estimate the hazard ratio (HR) of a spontaneous abortion with the underlying time scale being gestational age (in days). Time started at the index date (exposure date) and pregnant women only contributed to the risk time while being pregnant between the index date and week 22, thus pregnancies that were terminated prior (e.g., induced abortions) were censored'.

Reviewer comment 6) Discussion, line 247: please include the incidence of neonatal abstinence syndrome with tramadol exposure.

Authors reply 6) This is a very relevant comment; however, we have not been able to identify a study providing the relevant information. The incidence of neonatal abstinence syndrome (across all opioid exposures) has increased in the past decades[1]. This is presumably caused by an increase in opioid exposure among pregnant women. In 2017, the incidence of neonatal abstinence syndrome reached 7.3/1,000 neonatal hospitalizations[1]. In an article from 2016, it is stated that neonatal abstinence syndrome can occur in 55-94% of newborns whose mothers were addicted to or treated with opioids during pregnancy[2]. It would be extremely interesting in a future study to investigate the incidence of neonatal abstinence syndrome following tramadol exposure, possibly compared to morphine, oxycodone, and codeine exposure (as these are frequently used in Denmark).

Reviewer comment 7) Discussion, lines 249-253: Can you comment on who is missed by these registries? Those who experience spontaneous abortion at home without any hospital or office encounter would be missed. I do not believe these people would change your findings, but I think it is important to admit.

Authors reply 7) Thank you for this comment, which we have incorporated as a limitation in the discussion. Early spontaneous abortions taking place without a contact to the health care sector will not be identifiable in our study. If, hypothetically, there is an association between exposure to tramadol and very early spontaneous abortions, this would not be detectable in our study and would lead to a biased result. We have therefore added this to the revised manuscript on Page 12, Line 281: 'We only included registered pregnancies and thus, very early spontaneous abortions were potentially not included in the study. This might lead to a biased result if, hypothetically, there is an association between exposure to tramadol and very early spontaneous abortions'.

Reviewer #2:

This is a much needed study: the effects of many medications commonly prescribed to pregnant women are still poorly characterized. The authors undertook an important task: to characterize the structural effects on the developing fetus of a commonly used opioid, tramadol.

Reviewer comment 1) One concern I have is that most of the women in the study are on polytherapy with several other medications. and, while my concern is alleviated by the fact that women on tramadol tend to be on a similar or higher number of medications, we know that even within the same class of medications outcomes can vary greatly with different medications. However, given careful matching/selection of their cohorts, a stochastic distribution is likely. I would like the authors to report also the % MCM in those 653 pregnancies with exposure to tramadol monotherapy. That is a high enough number to provide valuable information, less tainted by other exposures.

Authors reply 1) Thank you very much for this relevant comment. We agree that a large proportion of tramadol-exposed women receive other medications. We have calculated the proportion of major congenital malformations among mono-tramadol-exposed and among unexposed women. We found that 20 children of 653 exposed pregnancies in total were diagnosed with a major congenital malformation within the first year after birth as compared to 84 children of 2,530 unexposed pregnancies. This corresponds to a RR of 0.92 (CI 95% 0.57-1.49) indicating no association corresponding with the original analysis. Hence, we have not included this in the manuscript but if reviewers and editors wish, we can add this to the manuscript.

Reviewer comment 2) If feasible, an analysis of dose-dependent effect in this monotherapy group would also be valuable.

Authors reply 2) We agree that this would be a valuable contribution, but considering the small sample size, we have abstained from conducting this analysis.

Reviewer comment 3) Another important observation is that the rate of major congenital malformations is higher than what is reported in the literature (1-3%) and closer to 4%. That is itself is a concern and, while the control cohort has a similar rate of MCMs, it is matched for the study cohort for comorbidities/medications. This may arguably suggest that perhaps tramadol exposure may be comparable with other medications in terms of risk of MCMs, given such high proportion of women on polytherapy in both of their cohorts, but that risk does seem higher than the risk published in the general population.

Authors reply 3) This is a relevant comment. When reading reported rates of major congenital malformations in the literature, they vary considerably[3,4]. This is probably due to differences in data sources, included populations, applied definitions, and reporting practices across countries and hospitals. In the original data set including all registered pregnancies in Denmark between 1997-2016, the rate of major congenital malformations is 3.4%. Our results are in line with this and previous studies using the same data sources[5]. Given these considerations, we do not consider the rates of major congenital malformations to be worrisome.

Reviewer comment 4) Most importantly, the authors should correct their conclusion in the body text: "we believe that use of tramadol during early pregnancy does not pose a risk for the foetus". I like better their conclusion in the abstract. They are not looking at all the risks an exposure can bring for the foetus, they are strictly looking at MCM and abortions. The exposure, even in the first trimester only, may be important for other neonatal outcomes (e.g. birth weight) and neurodevelopment.

Authors reply 4) Thank you for the observant comment which we strongly agree upon. We have adjusted the conclusion, so it is aligned in the abstract and the main text. It now reads on Page 12, Line 291: 'In this large nationwide cohort study, we found no association between exposure to tramadol during early pregnancy and an increased risk of spontaneous abortions or major congenital malformations'.

Reviewer comment 5) Of note, there is a redundancy in your introduction - Line 71-72 : "exposure to analgesics before a pregnancy is acknowledged is common"; "is acknowledged" should be removed.
Authors reply 5) We have corrected the sentence. It now reads on Page 4, Line 85: 'Pain and painful diseases are prevalent among women, and exposure to analgesics before a pregnancy is common'.
This is overall an excellent study.

Reviewer #3:

This study investigates the associations between early pregnancy exposure to tramadol, the most common prescription opioid in Denmark, and two outcomes, i.e., spontaneous pregnancy loss and congenital malformations, using data from the Danish National Registries (1997-2016). Propensity score matching was used to control for confounding. The investigators concluded that no associations were observed, although they admit that they cannot rule out the possibility of confounding by factors not available in the registry data, such as alcohol use.

There are strengths of the study, including its size and relatively complete data. The findings are assuring, given the difficulty for clinicians and patients to treat maternal pain symptoms while also safeguarding the health of the fetus. Still, I have several questions, most of which are clarifying the methods employed as well as disclosure of possible biases.

Reviewer comment 1) The paper describes the database as including "abortive outcomes with a hospital contact" (page 5 line 101). Does this mean that there may be spontaneous abortions excluded from the cohort if they did not involve a hospital visit? I understand why losses before 6 weeks were not included in the database. Still, I have some concern that the data may be affected by left truncation due to exclusion of some losses, as they may not be captured in the database. This is a potentially important limitation of the spontaneous loss findings, as left truncation, particularly if differential with respect to exposure status, can bias findings (Schisterman 2013).

Authors reply 1) Thank you for this comment, which we have incorporated as a limitation in the discussion. Early spontaneous abortions taking place at home without a contact to the health care sector were not identifiable in our study. If, hypothetically, there is an association between exposure to tramadol and very early spontaneous abortions, this would not be detectable in our study and would lead to a biased result. We have therefore added this to the revised manuscript on Page 12, Line 281: 'We only included registered pregnancies and thus, very early spontaneous abortions were potentially not included in the study. This might lead to a biased result if, hypothetically, there is an association between exposure to tramadol and very early spontaneous abortions'.

Reviewer comment 2) Please clarify why non-livebirths were excluded from congenital malformation analysis. Is it possible that this exclusion could have resulted in selection bias for certain subgroups of defects, as pregnancies may be terminated upon recognition of the malformation during obstetric ultrasound and some defects carry high risk of mortality (Heinke 2020a, Heinke 2020b)?
Authors reply 2) Thank you for this relevant comment. In Denmark, the incidence rates of stillbirths are stable around 0.3% per year, so based on a prespecified assumption of a small sample size, stillbirths as an outcome were not included as such an analysis would be limited with regards to power. In Denmark, the public health care sector offers two free, voluntary ultrasound examinations. In cases with a severe major congenital malformation, the woman has the possibility of a late elective termination. By law, this is after gestational age week 12, and these cases are registered in the nationwide healthcare registers. To address the specific issue with pregnancies being terminated upon recognition of a major congenital malformation, we conducted a sensitivity analysis on pregnancy terminations after 12 weeks to estimate terminations due to congenital malformations. These late terminations were approved in cases with a malformation.

Reviewer comment 3) The definition of the exposure classification and the possibility of misclassification warrant further discussion. The exposures were based on prescription data. Was the exposure period of interest (i.e., start of pregnancy through 12 weeks for malformations and start of pregnancy through 22 weeks for early losses) defined based on the prescription/fill date only, or based on the estimated period of use (based on number and frequency of doses covered by the prescription)?
Authors reply 3) Thank you for the comment on exposure definition which is relevant in all pharmacoepidemiologic studies and we apologize for the ambiguity. The exposure period was based solely on the date of filling the prescription at the pharmacy (the index date) and we did not estimate period of use. Unfortunately, we do not know if tramadol was prescribed for use on a daily basis or for sporadic use. We conducted a sensitivity analysis, which is not included in the manuscript due to the high number of sensitivity analyses, with the exposure period defined as filling a prescription from four month prior to conception and during gestational age 12 or 22 weeks depending on the outcome. The results of our analyses were unchanged compared with the original exposure definition.
We added the following to the discussion on Page 11, Line 278: 'We used redemption of a prescription as a proxy for use of tramadol. However, if tramadol was purchased but not ingested, or ingested outside the relevant exposure window, the exposure status would be misclassified. Further, it is a severe limitation that we were unable to discriminate between regular use and sporadic use'.

Reviewer comment 4) Also, did the prescriptions include both continuous and sporadic periods of use? If sporadic, and if the data are available, why wasn't the exposure analyzed at the day or week level? This seems particularly relevant for congenital malformations as the periods of organogenesis are very specific. Exposure as late as 11 or 12 weeks of gestation may be after onset of some malformations. The timing issue may also be relevant for studying the effect of tramadol on pregnancy loss, if the mechanism results from an acute exposure.

Authors reply 4) This is a very relevant comment; however also a common problem in pharmacoepidemiologic studies. We included both continuous and sporadic users, and we did not differentiate between the two patterns of use as our data were not fit for this discrimination. We assumed a stochastic distribution of the exposure dates based on our own previous analyses.

We tried to mimic a dose-response analysis to address a potential dose effect in our sensitivity analysis diving tramadol exposure into low dose (<5,000 mg) and high dose (≥5,000 mg). These results indicated no apparent association. We consider it a relevant limitation and have elaborated on it in the discussion on Page 11, Line 278: 'We used redemption of a prescription as a proxy for use of tramadol. However, if tramadol was purchased but not ingested, or ingested outside the relevant exposure window, the exposure status would be misclassified'.

We conducted an additional sensitivity analysis on the risk of a major congenital malformation during organogenesis, that is exposure from gestational week 4 to 10. Using this exposure period, the RR for a major congenital malformation when comparing tramadol exposure with no exposure was 0.91 (95% CI 0.72-1.14). If reviewers and editors wish, we can add this to the manuscript.

Reviewer comment 5) Since Cox proportional hazard models were used for the pregnancy loss analysis, it is unclear why time-varying exposure was not taken into account to evaluate this timing issue. Lastly, how were prescription/use of other types of opioids taken into account? Did you consider excluding women with prescriptions for other types of opioids or diagnosis of opioid use disorder to have a cleaner comparison? I raise these concerns because, while not always the case, non-differential misclassification can result in a bias towards the null, which may credibly explain the null findings without a more quantitative assessment of this form of bias

Authors reply 5) Thank you for the relevant comment. In the Cox proportional hazards regression model, we did account for the time factor. We agree on the uncertainty and have rephrased the section on Page 7, line 167: 'We used a Cox proportional hazards regression model to estimate the hazard ratio (HR) of a spontaneous abortion with the underlying time scale being gestational age (in days). Time started at the index date (exposure date) and pregnant women only contributed to the risk time while

being pregnant between the index date and week 22, thus pregnancies that were terminated prior (e.g., induced abortions) were censored'.
The confounder, opioid use, was included in the propensity score analysis and was well balanced in both cohorts. The mean standard difference was < 10%, however with more opioid users in the tramadol cohort. Therefore, the proportion using opioids is larger among the tramadol exposed. If it was a confounder then it would result in a larger risk estimate and hence away from a null finding.
We have conducted an additional sensitivity analysis with estimates for mono-tramadol exposure pregnancies. We found that 20 children of 653 exposed pregnancies in total were diagnosed with a major congenital malformation within the first year after birth as compared to 84 children of 2,530 unexposed pregnancies. This corresponds to a RR of 0.92 (CI 95% 0.57-1.49) indicating no association corresponding with the original analysis. Hence, we have not included this in the manuscript but if reviewers and editors wish, we can add this to the manuscript.

Minor Comments:

Reviewer comment 6) The paper lacks discussion of prior research on opioid use and risk of spontaneous abortions (e.g., Flannagan 2020). Is there reason to hypothesize the mechanism specific to tramadol would be different from other types of opioids?

Authors reply 6) Thank you for the comment and very interesting reference. Tramadol differentiates from the traditional opioids as it in addition to an affinity for the μ-opioid receptor inhibits the reuptake of noradrenaline and serotonin. We have added the following to the discussion: Page 10, Line 243: 'Knowledge on the risk of spontaneous abortion following tramadol exposure is relevant for both patients and physicians, and exposure to opioids during early pregnancy has been reported to increase the risk of a spontaneous abortion (Ref. Flannagan et al. 2020). Reassuringly, our results do not indicate an association between early exposure to tramadol and an increased risk of spontaneous abortion'.

Reviewer comment 7) How were correlations between multiples (e.g., twins) and between successive pregnancies of the same person over time handled in the analysis?

Authors reply 7) We have handled twins (and more) and multipara pregnancies as individual pregnancies in the analyses. We have, however, matched cases and controls on previous event of interest. When limiting the analysis to singleton and nullipara pregnancies, the estimates were similar to the main results with a RR for major congenital malformations of 1.09 (CI 95% 0.84-1.43).

Reviewer comment 8) Since variable ratio matching was applied, were the matching strata (e.g., 1:1 versus 1:3) taken into account in the analysis?

Authors reply 8) This is a relevant question. We predefined to use a matching ratio of up to 1:4 as previous studies have shown that matching up to 1:4 is optimal. It is possible to match using different ratios, 1:1, 1:3 etc. We prioritized optimal matching and some cases were only matched e.g. 1:3. However, we had extremely few cases that were not matched 1:4 and hence, if was deemed irrelevant to adjust for this in the analysis.

Reviewer comment 9) Page 6 lines 119-120 "Gestational age was generally calculated from the first day of the last menstrual period and subsequently confirmed by ultrasonography." For instances where this was not the case, how else was gestational age assigned?

Authors reply 9) In Denmark, the public health care sector offers two free, voluntary ultrasound examinations. The first takes place around gestational age 11 to 14 week, and the second around gestational age 18 to 20 week. If a woman does not attend the first ultrasound examination, the gestational age is calculated at the second ultrasound examination. If the woman does not attend any of the ultrasound examinations, the gestational age is calculated from the first day of the last menstrual period. In Denmark, the proportion of women attending the prenatal ultrasound examinations was 97.7% in 2013. We therefore do not consider it a mayor limitation in the study.

Reviewer comment 10) In the Methods, clarify what was done with medically induced abortions in the main models analyzing spontaneous losses.

Authors reply 10) We agree on the ambiguity in the method section. Pregnant women only contributed to the risk time while being pregnant between the index date and gestational age week 22. Thus, pregnancies that were terminated (e.g. induced abortions) prior to week 22 were censored. We have rephrased on Page 7, line 167: 'We used a Cox proportional hazards regression model to estimate the hazard ratio (HR) of a spontaneous abortion with the underlying time scale being gestational age (in days). Time started at the index date (exposure date) and pregnant women only contributed to the risk time while being pregnant between the index date and week 22, thus pregnancies that were terminated prior (e.g., induced abortions) were censored'.

Reviewer comment 11) What was the time scale used in the Cox models?

Authors reply 11) We chose the Cox proportional hazards regression model to account for the time factor. Pregnant women only contributed to the risk time while being pregnant between the index date

and gestational age week 22. Thus, pregnancies that were terminated (e.g. induced abortions) prior to week 22 were censored. We agree on the ambiguity and have rephrased the section on Page 7, line 167: 'We used a Cox proportional hazards regression model to estimate the hazard ratio (HR) of a spontaneous abortion with the underlying time scale being gestational age (in days). Time started at the index date (exposure date) and pregnant women only contributed to the risk time while being pregnant between the index date and week 22, thus pregnancies that were terminated prior (e.g., induced abortions) were censored'.

Reviewer comment 12) How were the malformation subgroupings defined (i.e., which defects comprised each subgroup)?

Authors reply 12) Information on major malformations was obtained from the Danish National Patient Registry and was based on diagnoses assigned by hospital physicians. The diagnoses were recorded during hospitalization and during the infants first year of life. We used the EUROCAT classification system version 1.4 to categories major malformations and subgroups. Subgroups of chromosomal disorders as well as other defects of known causes (e.g. Down syndrome and fetal alcohol syndrome) were excluded. We have added a table (supplementary Table 1) with ICD-10 codes used to identify major congenital malformations according to the EUROCAT 1.4 classification system.

Reviewer comment 13) Page 9 lines 205-206. "None of the risk estimates for subgroupings of major congenital malformations reached statistical significance" I recommend describing the effect estimates rather than solely relying on statistical significance. To quote the Journal's author guidelines "Putting the results in the form of an effect size makes the result of the statistical test more clinically relevant and gives better context" In particular, it appears that there may be something occurring with nervous system defects (RR 1.8, 95% CI 1.0, 3.6; Figure 3).

Authors reply 13) Thank you for this very relevant comment. We strongly agree that effect sizes are more valuable for the clinical interpretations than merely p-values or statements with 'statistical significance'. We admit that especially some of our sensitivity analyses have a low power – that is, we do not observe an association, but based on the study's limitations we cannot dismiss an association. We have made the following changes on Page 9, Line 223: 'Risk estimates from analyses of specific subgroupings of major congenital malformations identified no indication of an increased risk associated with tramadol exposure compared with no exposure (**Figure II**)' and on Page 12, Line 287: 'Lastly, due to low rates of events in subgroupings of major congenital malformations, the risk estimates should be interpreted with caution'.

Reviewer comment 14) In the Discussion the investigators noted "This study has several strengths including use of the Danish nationwide registries allowing for a detailed characterization of the population with minimal loss to follow-up." Were any participants excluded due to loss to follow-up? I don't recall seeing this noted in the eligibility flowchart.

Authors reply 14) Thank you for this observant comment which has led to a rephrasing in the discussion. By conducting analyses based on the Danish nationwide registers, selections bias and recall bias are eliminated and lost to follow-up minimized. In the discussion, it now states on Page 11, Line 269: 'This study has several strengths including use of the Danish nationwide registries. By conducting analyses based on these registers, selections bias and recall bias are eliminated'.

Reviewer comment 15) Page 11 line 252 If the congenital defects have a positive predictive value of 89%, as noted, wouldn't this imply that ~10% of infants classified as having a defect don't actually have a defect? Could this outcome misclassification contribute to a bias towards the null? Is outcome classification better for certain defect subgroups?

Authors reply 15) Thank you for this interesting comment. The positive predictive value (PPV) of registration is the most frequently reported measure of the validity of records in the DNPR. It is defined as the proportion of patients registered with a disease who truly have the disease and is usually estimated using medical record review as the reference standard to confirm the presence of disease. We agree on the risk of misclassification in our study. In a review by Schmidt et al., the PPV for certain malformation subgroupings is reported[6]. The PPV is e.g. 98.4% for cardiac malformations and 93.0% for hypospadias[6]. We anticipate that the risk of misclassification is non-differential, and hence can lead to a bias towards the null.

Reviewer comment 16) The following text is unclear: Page 4 lines 85-86 "Few studies have specifically assessed the risk of tramadol use in early pregnancy and these results have neither been conclusive." Is this meant to read "have been inconclusive"?

Authors reply 16) Thank you for this observant comment. We have corrected the sentence on Page 4, Line 98: 'Few studies have specifically assessed the risk of tramadol use in early pregnancy and these results have been inconclusive'.

Reviewer comment 17) Possible typos: Page 4 line 76 "200.000 adult users" should be "200,000 adult users"; Page 5 line 105 missing "materials" at the end of the following line "A description of the registers is provided in the supplementary."; Page 8 lines 189-190 "7,310 pregnancies were exposed to

tramadol at some time from conception and 22 weeks onwards" should instead end something like "from conception through 22 weeks"; Page 9 lines 200-201 "The majority (n=3,630) of the pregnancies was singleton pregnancies." should be "were singleton".

Authors reply 17) Thank you for your thoroughness while reviewing our manuscript. We have changed the following:

Page 4, line 89 now reads: 'In Denmark, tramadol is the most frequently used opioid with nearly 200,000 adult users in 2019'.

Page 5, line 120 now reads: 'A description of the registers is provided in the supplementary material'.

Page 9, lines 207 now reads: 'For the analyses on spontaneous abortions, we analysed 36,467 pregnancies of which 7,310 pregnancies were exposed to tramadol from conception through 22 weeks'.

Page 9, lines 218 now reads: 'The majority (n=3,630) of the pregnancies were singleton pregnancies'.

References:

Heinke D, Nestoridi E, Hernandez-Diaz S, Williams PL, Rich-Edwards JW, Lin AE, et al. Risk of stillbirth for fetuses with specific birth defects. Obstet Gynecol. 2020a;135(1):133-40. PMID: 31809437.

Heinke D, Rich-Edwards JW, Williams PL, Hernandez-Diaz S, Fisher SC, Desrosiers TA, et al. Quantification of selection bias in studies of risk factors for birth defects among livebirths. Paediatric and Perinatal Epidemiology. 2020b;34(6):655-64. PMID: 32249969.

Flannagan KS, Mumford SL, Siaarda LA, Radoc JG, Perkins NJ, Andriessen VC, Zolton JR, Silver RM, Schisterman EF. Is opioid use safe in women trying to conceive? Epidemiology. 2020;31(6):844-51. PMID: 33311959

Schisterman EF, Cole SR, Ye A, Plagtt RW. Accuracy loss due to selection bias in cohort studies with left truncation. Paediatric and Perinatal Epidemiology. 2013;27(5):491-502. PMID: 23930785.

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[1] Devlin LA, Young LW, Kraft WK, Wachman EM, Czynski A, Merhar SL, et al. Neonatal opioid withdrawal syndrome: a review of the science and a look toward the use of buprenorphine for affected infants. J Perinatol 2021;

[2] McQueen K, Murphy-Oikonen J. Neonatal Abstinence Syndrome. N Engl J Med 2016;375:2468–79.

[3] Corsello G, Giuffrè M. Congenital malformations. J Matern Fetal Neonatal Med 2012;25 Suppl 1:25–9.

[4] Broe A, Damkier P, Pottegård A, Hallas J, Bliddal M. Congenital Malformations in Denmark: Considerations for the Use of Danish Health Care Registries. CLEP 2020;12:1371–80.

[5] Andersson NW, Olsen RH, Andersen JT. Association between use of macrolides in pregnancy and risk of major birth defects: nationwide, register based cohort study. BMJ 2021;372:n107.

[6] Schmidt M, Schmidt SAJ, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449–90.

STATISTICS EDITOR COMMENTS:

Lines 40-46, Fig 2: While it is true that the rates of abortion and rates of major congenital malformations were similar, the studies were underpowered to to discern the differences found. For instance, for abortions, the HR = 1.06, with abortion rates of 12.2 in unexposed and 11.9 % in unexposed pregnancies. Using alpha = 0.05, power = 0.80 and the sample sizes at hand, the minimum detectable HR would be ~ 1.10. So, one can only conclude that the difference is not likely to exceed ~ 10%. For the analysis of major congenital malformations, the math is worse, since the samples and the rates of adverse events are both smaller. Applying the same sample size/ power criteria, the minimum detectable RR is ~ 1.25. For the individual subgroups of malformations, the math is much worse, again owing to the lower rates. Should simply report the rates of adverse outcomes along with relevant CIs, but cannot generalize that there is not a statistical difference, based on these data.

Author reply) Thank you for these very relevant comments. Compared with previous studies published on the matter, we have included a large cohort of pregnant women exposed to tramadol during early pregnancy. However, we agree with the limitations in power. The purpose of the study is not necessarily to detect whether the risk is increased by e.g. 3% or 5% but to estimate if a large risk can be ruled out. And this is in our opinion possible with the included study size. We have made corrections in the manuscript on Page 9, Line 223: 'Risk estimates from analyses of specific subgroupings of major congenital malformations identified no indication of an increased risk associated with tramadol exposure compared with no exposure (**Figure II**)' and on Page 12, Line 287: 'Lastly, due to low rates of events in subgroupings of major congenital malformations, the risk estimates should be interpreted with caution'.

EDITOR COMMENTS:

Please more explicitly speak to statistical power limitations, especially for the sub-group analyses.

Author reply) Thank you for the relevant comment. In line with comments from the statistics editor, we have rephrased in the result section on Page 9, Line 223: 'Risk estimates from analyses of specific subgroupings of major congenital malformations identified no indication of an increased risk associated with tramadol exposure compared with no exposure (**Figure II**)'. Further, in the discussion we have

added the following on Page 12, Line 287: 'Lastly, due to low rates of events in subgroupings of major congenital malformations, the risk estimates should be interpreted with caution.'

EDITORIAL OFFICE COMMENTS:

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

A. **OPT-IN: Yes, please publish my point-by-point response letter.** Yes, we agree on publishing the point-by-point response letter.
B. OPT-OUT: No, please do not publish my point-by-point response letter.

2. When you submit your revised manuscript, please make the following edits to ensure your submission contains the required information that was previously omitted for the initial double-blind peer review:
* Include your title page information in the main manuscript file. The title page should appear as the first page of the document. Add any previously omitted Acknowledgements (ie, meeting presentations, preprint DOIs, assistance from non-byline authors).
* Funding information (ie, grant numbers or industry support statements) should be disclosed on the title page and in the body text. For industry-sponsored studies, the Role of the Funding Source section should be included in the body text of the manuscript.
* Include clinical trial registration numbers, PROSPERO registration numbers, or URLs at the end of the abstract (if applicable).
* Name the IRB or Ethics Committee institution in the Methods section (if applicable).
* Add any information about the specific location of the study (ie, city, state, or country), if necessary for context.

3. Obstetrics & Gynecology uses an "electronic Copyright Transfer Agreement" (eCTA), which must be completed by all authors. When you uploaded your manuscript, each co-author received an email

with the subject, "Please verify your authorship for a submission to Obstetrics & Gynecology." Please check with your coauthors to confirm that they received and completed this form, and that the disclosures listed in their eCTA are included on the manuscript's title page.

Authors reply) This has been confirmed by co-authors.

4. If your study uses ICD-10 data, please make sure you do the following:

a. State which ICD-10-CM/PCS codes or algorithms were used as Supplemental Digital Content.

b. Use both the diagnosis and procedure codes.

c. Verify the selected codes apply for all years of the study.

d. Conduct sensitivity analyses using definitions based on alternative codes.

e. For studies incorporating both ICD-9 and ICD-10-CM/PCS codes, the Discussion section should acknowledge there may be disruptions in observed rates related to the coding transition and that coding errors could contribute to limitations of the study. The limitations section should include the implications of using data not created or collected to answer a specific research question, including possible unmeasured confounding, misclassification bias, missing data, and changing participant eligibility over time.

f. The journal does not require that the title include the name of the database, geographic region or dates, or use of database linkage, but this data should be included in the abstract.

g. Include RECORD items 6.3 and 7.1, which relate to transparency about which codes, validation method, and linkage were used to identify participants and variables collected.

Authors reply) We provide a flowchart of the study cohorts in Figure I and describe identification of the cohort from the registers on Page 5, Line 110. In the supplementary table 2, we describe from which data sources the different variables stem from. Further, in both the supplementary and the manuscript, we provide all codes used to identify exposure, outcomes, and covariates in the study.

5. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-

management/health-it-and-clinical-informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.
Authors reply) We have reviewed the definitions and find that our manuscript is written in accordance with the definitions.

6. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Original Research reports should not exceed 5,500 words; Case Reports should not exceed 2,000 words. Stated word limits include the title page, précis, abstract, text, tables, boxes, and figure legends, but exclude references.
Author reply) Our revised manuscript adheres to the space limitations.

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).
* If your manuscript was uploaded to a preprint server prior to submitting your manuscript to Obstetrics & Gynecology, add the following statement to your title page: "Before submission to Obstetrics & Gynecology, this article was posted to a preprint server at: [URL]."
Authors reply) Our revised manuscript adheres to the abovementioned guidelines.

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

Authors reply) A short title is presented on the Title Page.

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 limit for Original Research articles is 300 words. Please provide a word count.
Authors reply) Following revision, we have checked the abstract carefully, so it is aligned with the manuscript. A word count has been added to the Title Page.

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.
Authors reply) In our manuscript, we use the abbreviation HR for hazard ratio. This abbreviation is not listed in the abovementioned link, but we consider it to be an acknowledged and widespread abbreviation.

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.
Authors reply) When revising our manuscript, we have not encountered the use of '/' in sentences with words.

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%").

Author reply) We have revised our manuscript to emphasize the effect size and not whether the result is relevant in terms of statistical significance. We have made the following change on Page 9, Line 223: 'Risk estimates from analyses of specific subgroupings of major congenital malformations identified no indication of an increased risk associated with tramadol exposure compared with no exposure (**Figure II**).'

13. Line 224: Your manuscript contains a priority claim. We discourage claims of first reports since they are often difficult to prove. How do you know this is the first report? If this is based on a systematic search of the literature, that search should be described in the text (search engine, search terms, date range of search, and languages encompassed by the search). If it is not based on a systematic search but only on your level of awareness, it is not a claim we permit.

Authors reply) This is a very relevant comment, and we agree on editing the sentence. We did not perform a systematic search, but all authors are familiar and up to date with studies within the field. However, we acknowledge the lack of power to provide such a statement and have revised the discussion. On page 10, line 243 it now states: 'Knowledge on the risk of spontaneous abortion following tramadol exposure is relevant for both patients and physicians, and exposure to opioids during early pregnancy has been reported to increase the risk of a spontaneous abortion (Ref. Flannagan et al.). Reassuringly, our results do not indicate an association between early exposure to tramadol and an increased risk of spontaneous abortion'.

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

Authors reply) We have aligned the tables with the journal style and have changed the order of appearance of the abbreviations.

15. Please review examples of our current reference style at http://ong.editorialmanager.com (click on the Home button in the Menu bar and then "Reference Formatting Instructions" document under

"Files and Resources). Include the digital object identifier (DOI) with any journal article references and an accessed date with website references. Unpublished data, in-press items, personal communications, letters to the editor, theses, package inserts, submissions, meeting presentations, and abstracts may be included in the text but not in the reference list.

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Authors reply) We have updated our reference style to comply with the current reference style.

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Authors reply) We submitted the two figures as PowerPoint files as these are the original files.

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