PEER REVIEW HISTORY

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ARTICLE DETAILS

<table>
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<th>TITLE (PROVISIONAL)</th>
<th>Safety of Influenza Vaccination During Pregnancy: A Systematic Review</th>
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<tr>
<td>AUTHORS</td>
<td>Wolfe, Dianna; Fell, Deshayne; Garrity, Chantelle; Hamel, Candyce; Butler, Claire; Hersi, Mona; Ahmadzai, Nadera; Rice, Danielle; Esmaeilsaraji, Leila; Michaud, Alan; Soobiah, Charlene; Ghassemi, Marco; Khan, Paul; Sinilait, Angela; Skidmore, Becky; Trico, Andrea; Moher, David; Hutton, Brian</td>
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VERSION 1 – REVIEW

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<tr>
<th>REVIEWER</th>
<th>Pedro L. Moro</th>
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<td>Centers for Disease Control and Prevention</td>
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<td>REVIEW RETURNED</td>
<td>06-Oct-2022</td>
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GENERAL COMMENTS

This is a review article and meta-analysis of the safety of influenza vaccines in pregnancy looking at 63 observational studies of seasonal influenza vaccine studies and pandemic vaccines that were retrieved from electronic databases. The meta-analyses for some condition such as preterm birth, spontaneous abortion and SGA did not identify any significant association. The authors found that seasonal influenza vaccination during pregnancy was not associated with adverse birth outcomes or maternal non-obstetric adverse events. The findings of this study are important and provide important information on the safety of influenza vaccine in pregnancy and point to some methodological issues and limitation with some of the studies reviewed. This will be an article of great interest to vaccinologists and public health professionals. I recommend its publication.

A few suggestions are noted below.

Comments:
In the methods of the abstract the authors should include the date period of the studies they included. They should point out any major exclusions.
Although I understand the intention was to do a meta-analysis the authors could have also included in a separate section the findings of RCTs which they decided to exclude from the study.

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<th>REVIEWER</th>
<th>Alberto Donzelli</th>
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<td>Fondazione Allineare Sanità e Salute</td>
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<td>REVIEW RETURNED</td>
<td>27-Feb-2023</td>
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GENERAL COMMENTS

Reviewer of “Safety of Influenza Vaccination During Pregnancy: A Systematic Review”

This systematic review aims to evaluate associations between influenza vaccination during pregnancy and adverse birth outcomes.
and maternal non-obstetric serious adverse events. The objective is relevant, because the WHO has recommended vaccination of pregnant women since 2005, without robust evidence from RCTs of its safety, although a necessary condition for universal vaccination should be its proven safety, beyond any doubt. The authors stated to have excluded RCTs for this review of safety outcomes, “given the rarity of most birth outcomes of interest and given the different settings of RCTs (lower-to-middle-income countries) on this topic compared to observational studies (high-income countries) that would increase clinical and statistical heterogeneity.”

However, this choice is not acceptable, for two major reasons:
1) The observational design is subject, among others, to the “healthy adherer bias”: subjects who adhere to preventive therapies are at the same time more likely to engage in healthy lifestyles than patients not adhering to such strategies. A healthy lifestyle includes diet, exercise, less alcohol intake and risky behaviors, and the search for better health care. The adherence to vaccination can also be linked to more trust in the proposed intervention, and this in turn might lead to better outcomes to some extent. Some of these characteristics are not measured in pharmacological databases, or are difficult to capture even when applying a propensity score weighting, because it is impossible to include unmeasured or unknown confounders in the propensity scores.

The healthy-adherer bias may be strong in pregnant women: more self-disciplined and educated women generally have healthier behaviors and are more adherent to recommendations by physicians, obstetricians, and health authorities, making them much more likely to receive vaccination, unlike socio-economically and culturally less advantaged women. Quite likely, the worse socio-economic and behavioral conditions of unvaccinated women could explain the worse outcomes of their children, without having to appeal to the explanation of missed vaccination.

2) Moreover, some good and large RCTs are currently available. After a first small RCT in Bangladesh, the Bill & Melinda Gates Foundation funded three large blinded RCTs, also to overcome the validity issues of observational evidence.

One, the Matflu, was in an upper-middle-income country (South Africa). It is considered “at low risk of bias” by Cochrane reviewers (Demicheli et al., 2018), with a number to vaccinate (NNV) 55 to avoid one influenza in mothers. Two other RCTs were in low-income countries (Mali, Nepal), where the expected benefits were greater. Besides the placebo-controlled Matflu RCT, included in the Cochrane review, one can also take into account the placebo-controlled Nepalese RCT, in which the NNV (not specified) seems about 20; and the larger RCT in Mali, NNV 99 (with a control group injected with quadrivalent meningococcal vaccine). The overall NNV is not far from the Cochrane estimate, close to the NNV for healthy adults.

In addition to a “very modest” efficacy, according to the best evidence considered by the Cochrane reviewers (Demicheli, et al., 2018), the placebo controlled trials showed an excess of maternal local adverse effects. Moreover, in the influenza-vaccinated women the offspring mortality tended to be higher than in the control groups. The overall serious adverse events (SAEs) tended to be more numerous, as summarized in detail in https://www.mdpi.com/1660-4601/16/22/4347. In the larger RCT the excess of SAEs in the offspring was statistically significant, RR 1.27; CI 95% 1.05–1.53; NNH (number needed to harm) 42.98. A sensitivity analysis excluding from the SAE count the major congenital malformations,
again shows a significant excess of SAEs in the vaccinated group: RR 1.27; CI 95% 1.05–1.53; NNH 44.80. For a comprehensive review of the safety outcomes of the above RCTs, see https://www.mdpi.com/1660-4601/16/22/4347, Table 1. Therefore, since observational studies in pregnant women (usually quite young and healthy) are prone to an important healthy-vaccinee bias, to promote a preventive pharmacological intervention, particularly in the vulnerable period of a pregnancy, public health services should not rely primarily on observational evidence (and more so only on observational evidence). Even more so if the (insufficient) safety evidence from the existing RCTs shows a tendency to move in an opposite and alarming direction. To evaluate the safety of influenza vaccination during pregnancy there is no need of another systematic review of observational studies (moreover, not taking into account the already available evidence from RCTs). Such a review not only does not add any real value, but it contributes to induce and reinforce a safety perception not legitimized by the available data. It would even contribute to preclude a further large, independent, and possibly reassuring RCT in high-income countries, to overcome the safety signals raised by the RCTs today available.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1 Comments

Dr. Pedro L. Moro, Centers for Disease Control and Prevention

Comments to the Author:

This is a review article and meta-analysis of the safety of influenza vaccines in pregnancy looking at 63 observational studies of seasonal influenza vaccine studies and pandemic vaccines that were retrieved from electronic databases. The meta-analyses for some conditions such as preterm birth, spontaneous abortion and SGA did not identify any significant association. The authors found that seasonal influenza vaccination during pregnancy was not associated with adverse birth outcomes or maternal non-obstetric adverse events. The findings of this study are important and provide important information on the safety of influenza vaccine in pregnancy and point to some methodological issues and limitation with some of the studies reviewed. This will be an article of great interest to vaccinologists and public health professionals. I recommend its publication. A few suggestions are noted below.

1. In the methods of the abstract the authors should include the date period of the studies they included. They should point out any major exclusions.

   • Authors’ Response: Thank you for this suggestion, we have added the date of the last search update to the abstract and the date range of included studies to the Results section (last line of Study characteristics). A line has been added to the abstract regarding major exclusions for study eligibility.

2. Although I understand the intention was to do a meta-analysis the authors could have also included in a separate section the findings of RCTs which they decided to exclude from the study.

   • Authors’ Response: We thank the author for this suggestion. While we have maintained our decision not to formally include RCTs in the review for several reasons (i.e., RCTs are insufficiently powered for safety outcomes and available RCTs are poorly generalizable, given LMIC settings and year-round influenza seasons), we have taken efforts in our revised manuscript to provide a detailed description of two recent pooled analyses of safety data of the relevant RCTs (i.e., Omer et al., 2020 and Regan and Munoz, 2021) within our discussion section to provide this context. We felt this a helpful way to provide the RCT context given that these syntheses have been recently published (we have also noted this within our methods section with the following sentence: “All birth outcome data from RCTs have previously been pooled in other recent publications23,24 and are summarized in the Discussion.”
Reviewer 2 Comments
Dr. Alberto Donzelli, Fondazione Allineare Sanità e Salute Comments to the Author:

- Authors’ Response: We thank Reviewer 2 for these detailed comments. We have attempted to respond within the flow of the reviewer’s text below.

This systematic review aims to evaluate associations between influenza vaccination during pregnancy and adverse birth outcomes and maternal non-obstetric serious adverse events. The objective is relevant, because the WHO has recommended vaccination of pregnant women since 2005, without robust evidence from RCTs of its safety, although a necessary condition for universal vaccination should be its proven safety, beyond any doubt. The authors stated to have excluded RCTs for this review of safety outcomes, “given the rarity of most birth outcomes of interest and given the different settings of RCTs (lower-to-middle-income countries) on this topic compared to observational studies (high-income countries) that would increase clinical and statistical heterogeneity.”

However, this choice is not acceptable, for two major reasons:
1) The observational design is subject, among others, to the “healthy adherer bias”: subjects who adhere to preventive therapies are at the same time more likely to engage in healthy lifestyles than patients not adhering to such strategies. A healthy lifestyle includes diet, exercise, less alcohol intake and risky behaviors, and the search for better health care. The adherence to vaccination can also be linked to more trust in the proposed intervention, and this in turn might lead to better outcomes to some extent. Some of these characteristics are not measured in pharmacological databases, or are difficult to capture even when applying a propensity score weighting, because it is impossible to include unmeasured or unknown confounders in the propensity scores. The healthy-adherer bias may be strong in pregnant women: more self-disciplined and educated women generally have healthier behaviors and are more adherent to recommendations by physicians, obstetricians, and health authorities, making them much more likely to receive vaccination, unlike socio-economically and culturally less advantaged women. Quite likely, the worse socio-economic and behavioral conditions of unvaccinated women could explain the worse outcomes of their children, without having to appeal to the explanation of missed vaccination.

- Authors’ Response: We thank the reviewer for sharing these perspectives on the challenges of observational data in the field. Regarding unmeasured confounders in observational studies, in the current review we have made considerable effort to address this both through the methodology used (e.g., only including adjusted estimates in meta-analyses) and in our discussion of confounding in both the Introduction and Discussion sections. While we did not exclude studies from meta-analyses or summaries if they did not adjust for socio-economic status (SES), of all studies in all reported syntheses of prioritized outcomes, only one (Panagiotakopoulos et al., 2020) did not control for SES and it was descriptively summarized and not included in a meta-analysis. We have added the following sentence to the end of the Study characteristics subsection of the Results: “All 15 studies included in the prioritized syntheses reported below adjusted for critical confounders (i.e., maternal age, smoking during pregnancy, and, socioeconomic status (SES)), except three cohort studies evaluating SGA that did not adjust for smoking during pregnancy64 96 99 and one case-control study evaluating stillbirths that did not adjust for SES122.” As well, we have added text in the SGA and stillbirth subsections of the Results section that identifies studies that did not control for all critical confounders. We feel it is important to note that, to date, this review is the only SR to address both confounding and immortal time bias in a rigorous manner, and thus we feel it can make an important addition to the literature. We have mentioned this important point within the second paragraph of the discussion section in stating: “To the best of our knowledge, no previous review has attempted to reduce temporal and confounding biases.”

2) Moreover, some good and large RCTs are currently available. After a first small RCT in Bangladesh, the Bill & Melinda Gates Foundation funded three large blinded RCTs, also to overcome the validity issues of observational evidence.

One, the Matflu, was in an upper-middle-income country (South Africa). It is considered “at low risk of bias” by Cochrane reviewers (Demicheli et al., 2018), with a number to vaccinate (NNV) 55 to avoid
one influenza in mothers. Two other RCTs were in low-income countries (Mali, Nepal), where the expected benefits were greater. Besides the placebo-controlled Matflu RCT, included in the Cochrane review, one can also take into account the placebo-controlled Nepalese RCT, in which the NNV (not specified) seems about 20; and the larger RCT in Mali, NNV 99 (with a control group injected with quadrivalent meningococcal vaccine). The overall NNV is not far from the Cochrane estimate, close to the NNV for healthy adults. In addition to a "very modest" efficacy, according to the best evidence considered by the Cochrane reviewers (Demicheli et al, 2018), the placebo controlled trials showed an excess of maternal local adverse effects.

- Authors’ Response: We appreciate the reviewers’ views of recent RCTs, though have some perspectives we feel are also worthy of mention. Regarding the Matflu study, while we agree that South Africa is a UMIC, the trial conducted by Madhi et al. was situated in Soweto, one of the most impoverished townships in the world, with very high rates of unemployment. Given the context, this study is not generalizable to HICs. Additionally, regarding its risk of bias from Demicheli et al, we assessed this study to have an unclear risk of bias due to unclear allocation concealment (it was not reported if envelopes were sealed and opaque) and unclear selective outcome reporting (several secondary outcomes and time points reported in the protocol were not reported in the final publication). These differ from Demicheli et al., who did not comment on the integrity of the envelopes used for assignment concealment and did not assess selective outcome reporting. Regarding the additional studies mentioned from Nepal and Mali, while we agree with Dr. Donzelli’s comments regarding efficacy reported in the RCTs, our review focused on influenza vaccine safety not efficacy. These studies were not powered to address safety outcomes, and were not generalizable due to context (i.e., SES and year-round influenza seasons).

In addition to a “very modest” efficacy, according to the best evidence considered by the Cochrane reviewers (Demicheli et al., 2018), the placebo-controlled trials showed an excess of maternal local adverse effects. Moreover, in the influenza-vaccinated women the offspring mortality tended to be higher than in the control groups. The overall serious adverse events (SAEs) tended to be more numerous, as summarized in detail in https://can01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.mdpi.com%2F1660-4601%2F16%2F22%2F4347&data=05%7C01%7C7Cwolfe%40ohri.ca%7C60d4506010184f5b294908db3c262cb4%7C6130f4d13a6931f6e4e7cb5a%7C0%7C7C638169906869214925%7CUunknown%7C7WPFpbGZbs3d8eyJWljoiMC42LiAwMDAIlCJQioiV2VuMUZileLCJBTiI6ik1haWwlLCJXVCI6Mn0%3D%7C3000%7C7C%7C7C&sdata=nGVmxb1SF%2Fk2RnSQxF9zO4v68PjX9Xim6%2FrXrBG

- Authors’ response: We thank the author for sharing these detailed data, though we find a different interpretation in reviewing these data. In the only RCT included in the Cochrane review by Demicheli et al (i.e., Madhi et al), early infant deaths up to 7 days were the same in both vaccinated and placebo groups (0.9 vs 0.9%) and infant deaths up to 6 months were higher in the control group (1.5% vs 2%). In reading the review, it would appear no comments regarding local reactions in pregnant women were made by Demicheli et al. Regarding SAEs and hospitalizations, Madhi states “In all cases, a biological cause unrelated to the vaccine was identified,” and regarding maternal deaths, “None of the stillbirths, miscarriages, newborn deaths or maternal deaths was attributed to IIV3-vaccination.” Therefore, causal assessment of adverse events by the study authors determined no associations with the interventions.

In the larger RCT [i.e., by Tapia et al], the excess of SAEs in the offspring was statistically significant, RR 1.27; CI 95% 1.05–1.5; NNH (number needed to harm) 42.98. For a sensitivity analysis excluding from the SAE count the major congenital malformations, again shows a significant excess of SAEs in the vaccinated group: RR 1.27; CI 95% 1.05–1.53; NNH 44.80. For a comprehensive review of the safety outcomes of the above RCTs, see Table 1 from: https://can01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.mdpi.com%2F1660-4601%2F16%2F22%2F4347&data=05%7C01%7C7Cwolfe%40ohri.ca%7C60d4506010184f5b294908db3c262cb4%7C6130f4d13a6931f6e4e7cb5a%7C0%7C7C638169906869214925%7CUunknown%7C7WPFpbGZbs3d8eyJWljoiMC42LiAwMDAIlCJQioiV2VuMUZileLCJBTiI6ik1haWwlLCJXVCI6Mn0%3D%7C3000%7C7C%7C7C&sdata=nGVmxb1SF%2Fk2RnSQxF9zO4v68PjX9Xim6%2FrXrBG

Bmj%3D&reserved=0.
• Authors’ Response:
  o We again very much thank the author for these detailed comments. Very respectfully, we do wish to flag a concern regarding the calculations provided by the reviewer. The infant SAEs reported in the trial by Tapia et al. were stated not to be mutually exclusive (i.e., meaning more than one event could occur in one infant). Therefore, risk of additional events would be higher in infants experiencing one event (e.g., the risk of death would be higher in infants with major congenital malformations), meaning events may be clustered at the infant level. We are concerned that the approach of summing individual events (as was presented in the reviewer’s stated personal calculations within Section 3.2 of the above indicated published communication) is not the appropriate technique to calculate an overall RR. A Poisson model on the individual-level patient data would be the only way of determining differences in event rates across groups. Alternatively, infant risk could be compared between groups using dichotomous measures of presence/absence of at least one SAE in the live births. However, neither individual patient data nor dichotomous presence/absence data are available from the publication upon which an RR could be correctly estimated.
  o Regarding the data and conclusions reported by Tapia et al., there were no differences in maternal non-obstetric SAEs (14/2,108 vs 14/2,085), severe systemic reactions within 7 days post-vaccination (2/2,105 vs 0/2,082), or severe local reactions within 7 days (1/2,105 vs 4/2,082), and the authors state in the Results (page 1033), “No serious adverse event was related to study treatment.”

Therefore, since observational studies in pregnant women (usually quite young and healthy) are prone to an important healthy-vaccinee bias, to promote a preventive pharmacological intervention, particularly in the vulnerable period of a pregnancy, public health services should not rely primarily on observational evidence (and more so only on observational evidence). Even more so if the (insufficient) safety evidence from the existing RCTs shows a tendency to move in an opposite and alarming direction. To evaluate the safety of influenza vaccination during pregnancy there is no need of another systematic review of observational studies (moreover, not taking into account the already available evidence from RCTs). Such a review not only does not add any real value, but it contributes to induce and reinforce a safety perception not legitimized by the available data. It would even contribute to preclude a further large, independent, and possibly reassuring RCT in high-income countries, to overcome the safety signals raised by the RCTs today available.

• Authors’ Response: We thank the reviewer for these thoughts. Within the revised manuscript we have (1) presented justifications for not including RCTs in our methods; (2) as noted earlier in response to Reviewer 1, we have updated the Discussion to include a summary of two recent pooled analyses of the RCTs in question (Omer et al 2020, Regan & Munoz 2021) to provide insights regarding the RCT results, as suggested by both reviewers. In doing so, we show that our findings from meta-analysis are similar to those of pooled RCT data recently published, without introducing clinical heterogeneity into our review, related to the year-round influenza seasons and lower socioeconomic conditions present in the available RCTs. As well, none of the available RCTs are generalizable to HICs and none demonstrate a low ROB, when fully assessed. In alignment with the recent descriptive synthesis of maternal adverse events following influenza vaccination by Regan and Munoz, we respectfully disagree with the reviewer’s interpretation of safety data reported in the available RCTs.