Maternal Influenza Immunization and Birth Outcomes of Stillbirth and Spontaneous Abortion: A Systematic Review and Meta-analysis

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Despite strong evidence that maternal influenza vaccination during pregnancy is safe, uptake of influenza vaccination during pregnancy remains low. We identified studies that assessed outcomes of stillbirth or spontaneous abortion after administration of influenza vaccine during pregnancy. We conducted a literature search in November 2013 that yielded 447 total citations. After removal of duplicates and studies deemed not relevant based on the title and abstract, 36 records underwent a full text review and 7 studies were included in the final review. Where possible, adjusted results were included in the meta-analysis. Women in the influenza vaccine group had a lower likelihood of stillbirth (relative risk [RR], 0.73; 95% confidence interval [CI], .55–.96); this association was similar when restricted to the H1N1pdm09 vaccine (RR, 0.69; 95% CI, .53–.90). The pooled estimate for spontaneous abortion was not significant (RR, 0.91; 95% CI, .68–1.22). These analyses add to the evidence base for the safety of influenza vaccination in pregnancy.

Keywords. influenza vaccine; pregnancy; stillbirth; spontaneous abortion.

Pregnant women are at an especially high risk of influenza-associated morbidity and mortality [1–4]. As a result, the inactivated influenza vaccine has been considered an element of prenatal care since the 1950s and has been recommended during any trimester of pregnancy since the mid-2000s [5–7]. The benefits of antenatal immunization have recently been demonstrated to extend beyond preventing influenza episodes in mothers: A randomized controlled trial in Bangladesh reported a significant reduction in influenza burden in infants of women vaccinated during pregnancy [8], and other studies have reported a lower proportion of infants born premature or small for gestational age (SGA) among women vaccinated against influenza antenatally [9–11].

Despite studies that have provided evidence that there is a null, if not protective, association between maternal influenza vaccination and adverse fetal and neonatal outcomes, concerns regarding the safety of influenza vaccination during pregnancy persist. In the United States and Canada, misconceptions about the risk of influenza vaccination during pregnancy and concerns about safety of the influenza vaccine are commonly cited reasons for suboptimal uptake of influenza vaccination in pregnancy [12, 13]. Evidence that antenatal immunization is safe in terms of adverse birth outcomes may prove important in increasing coverage of influenza vaccine in this high-risk group.

Outcomes of stillbirth and spontaneous abortion following maternal receipt of influenza vaccination have been less studied than outcomes of preterm birth, low birth weight, and SGA birth. A putative biological mechanism for the relationship between influenza
METHODS

Study Selection
We sought studies assessing the association between influenza vaccination during or prior to pregnancy on birth outcomes of spontaneous abortion and stillbirth. Relevant articles considered an exposure to seasonal or H1N1pdm09 influenza vaccination in any stage of pregnancy or immediately prior to conception and measured an outcome of either spontaneous abortion or stillbirth. Outcomes must have been measured in both a vaccinated and unvaccinated group (studies that used surveillance data compared to population rates of birth outcomes were ineligible). After the literature search process was complete, we restricted the analysis to studies that defined spontaneous abortion as fetal loss at gestational age <20 weeks and stillbirth as fetal loss at ≥20 weeks, the definitions used by the American Congress of Obstetricians and Gynecologists. Studies that defined the cutoff at 22 weeks were also included.

Only articles published in peer-reviewed journals were eligible for inclusion. Truncated abstracts, books, professional and clinical guidelines and recommendations, and reviews were excluded, as were immunological, pharmacological, and nonhuman studies. All study designs were eligible for inclusion.

Search Strategy and Selection Criteria
A systematic literature search was conducted in November 2013 using keyword terms to identify relevant articles in the following electronic databases: Medline (PubMed and OVID search engines), Embase, Web of Science, Cumulative Index to Nursing or Allied Health Literature, Scopus, Google Scholar, and Cochrane Central Register of Controlled Trials. Search terms included the Medical Subject Heading (MesH) terms stillbirth, spontaneous abortion, influenza vaccination, and influenza vaccines, as well as the terms influenza vaccin*, influenza immuniz*, flu vaccin*, flu immuniz*, and perinatal death in search engines that did not use MesH terms.

The titles and abstracts of each article were reviewed against inclusion criteria. References of articles found in initial searches were used to locate additional relevant studies; these studies were also subjected to a title and abstract review. Relevant articles were retained and the full texts of these articles were reviewed again for eligibility. After the full text review, articles meeting the predefined inclusion criteria were included in the final review.

Data Abstraction
Data abstraction was performed for each article, using a tool created based on recommendations from the Cochrane Handbook for Systematic Reviews of Interventions version 5.1.0 [14] and the Methods Guide for Effectiveness and Comparative Effectiveness Reviews, created collaboratively by the Effective Health Care (EHC) Program, the Agency for Healthcare Research and Quality (AHRQ), the EHC Program Scientific Resource Center, and the AHRQ Evidence-Based Practice Centers [15]. For each included study, information abstracted included study design and research methods, study subject characteristics, measures of association and precision, and, if applicable, for which variables the measure was adjusted. There were 5 domains of bias for which each individual study was evaluated: selection, performance, attrition, reporting, and detection bias. Information abstracted to assess risk of bias included recruitment and selection procedures for participants and methods for control of confounding (selection bias), likelihood of concurrent interventions and fidelity to the intervention protocol (performance bias), methods for handling missing data (attrition bias), procedures for exposure, outcome, and confounder ascertainment (detection bias), and full reporting of all prespecified outcomes (reporting bias). Based on this information, the potential for bias in each domain was expressed using a risk of bias score of “low” or “high.” Domains for which not enough information regarding the methods was available were assigned an “unclear” risk of bias. Extraction and bias assessment were performed by a second reviewer to ensure accuracy of data collection and risk of bias score assignment. Inconsistencies were assessed and resolved by the first reviewer.

Statistical Analyses
Forest plots were constructed to depict the association between birth outcomes and all vaccination (H1N1pdm09 and nonpandemic seasonal) or H1N1pdm09-only vaccination. Ratio measures and confidence intervals (CIs) were calculated using OpenEpi [16] for studies that did not calculate ratio measures but reported the necessary information. Unadjusted and adjusted measures of association were included in pooled estimates. Hazard ratios (HRs) and relative risks (RRs) were considered comparable, as they were both estimated at the completion of the study after the risk period for the outcome had ended. Heterogeneity of effect measures, including I² and the corresponding P value, were calculated with log relative effect measures using a random-effects model. Summary measures were calculated using the DerSimonian and Laird method [17] for subgroups in which there was >1 study reporting ratio-based measures. Funnel plots were constructed to detect the existence of publication bias. All meta-analysis statistical procedures were performed using Stata software, version 13.1.
RESULTS

Literature Search Results
Electronic literature searches yielded 447 total citations. After 95 duplicates were removed, 359 more studies were removed based on a title or abstract that was deemed not relevant based on inclusion criteria, and the 29 remaining records underwent a full text review. Seven more studies were located through manual searching of the cited references from these 29 articles. Of these 36 studies, 29 were removed because the exposure or outcome measures did not meet inclusion criteria, and 7 studies were included in the final review (Figure 1).

Of these 7 studies, 2 were retrospective cohort studies, 4 were prospective cohort studies, and 1 was a cross-sectional study. Six studies assessed outcomes associated with H1N1pdm09 vaccine receipt, and 1 study assessed 1976 monovalent H1N1 (A/NJ/8/76) vaccine receipt (Table 1). For purposes of analysis, we categorized studies into 3 groups according to vaccine type: (1) all influenza vaccines (7 studies); (2) H1N1pdm09 vaccine (6 studies), and (3) nonpandemic monovalent H1N1 influenza vaccine (1 study). These categories were not mutually exclusive; for example, the study of the 1976 monovalent H1N1 vaccine (A/NJ/8/76) would fall into the first and third groups. Three of 7 studies measuring outcomes of stillbirth and 3 of 4 studies...
measuring outcomes of spontaneous abortion reported effect estimates adjusted for potentially confounding factors.

**Overall Impact of Influenza Vaccination**

Overall, women in the influenza vaccine group had a lower likelihood of stillbirth (RR, 0.73 [95% CI, 0.55–0.96]; Figure 2A). Of the 7 individual studies that assessed the impact of any type of vaccination on stillbirth, 4 studies found relative effect measures <1, and the 2 studies reporting significant relative effect measures were the largest studies. Fell et al reported an adjusted RR of 0.66 (95% CI, 0.47–0.91) [18] for stillbirth, defined as fetal loss at ≥20 weeks of gestation, and Pasternak et al reported an adjusted HR of 0.44 (95% CI, 0.20–0.94) [19] for stillbirth, defined as fetal loss at ≥22 weeks of gestational age. Five studies found that there was no association between influenza vaccination and stillbirth rates and reported RRs of 1.10 (95% CI, 0.47–2.57) [20], 2.74 (95% CI, 1.7–43.5) [21], and 0.23 (95% CI, 0.1–3.93) [22], with stillbirth defined as fetal loss at gestational age >20 weeks, and adjusted odds ratios of 1.44 (95% CI, 0.23–8.90) [23] and 0.72 (95% CI, 0.47–1.11) [24], with stillbirth defined as loss after 22 weeks (Figure 2A). A sensitivity analysis excluding studies with a high risk of bias in >1 domain showed a similar overall relationship (RR, 0.69 [95% CI, 0.53–0.90]; Supplementary Figure 1).

The pooled effect of any vaccination on spontaneous abortion was nonsignificant (RR, 0.91 [95% CI, 0.68–1.22]; Figure 2B). Of the 5 studies that assessed the impact of any type of vaccination on spontaneous abortion, 4 found null associations. These studies reported risk ratios of 0.60 (95% CI, 0.22–1.63) [20] and 0.91 (95% CI, 0.19–4.48) [21] and an adjusted HR of 0.92 (95% CI, 0.31–2.72) [22], with spontaneous abortion defined as fetal loss prior to 20 weeks of gestational age, and an adjusted HR of 1.11 (95% CI, 0.71–1.73) [19], with spontaneous abortion defined as fetal loss from 7 to 22 weeks of gestational age. The last study that measured spontaneous abortion observed no events in the vaccinated group; therefore, no measures of association were calculated [23] (Figure 2B).

Statistical tests showed significant heterogeneity for the pooled effects of H1N1pdm09 and overall vaccination on stillbirth but no significant heterogeneity for pooled estimates of spontaneous abortion (Figure 2A–D).

**H1N1pdm09 Vaccination**

The association between H1N1pdm09 vaccination and stillbirth was similar to that of overall vaccination (RR, 0.69 [95% CI, 0.53–0.90]), as 6 of the 7 studies that assessed the relationship between maternal influenza vaccination and stillbirth examined
Figure 2. A–D, Forest plots for stillbirth and spontaneous abortion, overall and H1N1pdm09 only. The center of each box represents the point estimate of effect reported for each study, and arrows represent 95% confidence intervals (CIs) extending outside of the shown range. The size of the gray-shaded boxes corresponds to the weight of the study in the meta-analysis (larger studies weighted more heavily). The dotted lines and diamonds indicate the pooled point estimate and corresponding CI, respectively.
Table 2. Associations Between Maternal Influenza Vaccination and Stillbirth

<table>
<thead>
<tr>
<th>Study</th>
<th>Vaccine Type</th>
<th>No. Vaccinated (No. Stillbirth Events)</th>
<th>No. Unvaccinated (No. Stillbirth Events)</th>
<th>Measure</th>
<th>Effect (95% CI)</th>
<th>Factors Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chambers et al [22]</td>
<td>H1N1pdm09</td>
<td>191 (1)</td>
<td>338 (1)</td>
<td>Relative risk, first trimester</td>
<td>0.57 (.03–9.57)</td>
<td>. . .</td>
</tr>
<tr>
<td>Cantu et al [20]</td>
<td>H1N1pdm09</td>
<td>979 (8)</td>
<td>2010 (15)</td>
<td>Relative risk, any trimester</td>
<td>0.23 (.01–3.93)</td>
<td>. . .</td>
</tr>
<tr>
<td>Deinard et al [21]</td>
<td>A/NJ/876 H1N1</td>
<td>189 (1)</td>
<td>517 (1)</td>
<td>Relative risk</td>
<td>2.74 (.17–43.5)</td>
<td>Maternal age, family income, education</td>
</tr>
<tr>
<td>Fell et al [18]</td>
<td>H1N1pdm09</td>
<td>23340 (60)</td>
<td>32230 (139)</td>
<td>Adjusted relative risk</td>
<td>0.66 (.47–91)</td>
<td>Parity, smoking, maternal age</td>
</tr>
<tr>
<td>Heikkinen et al [23]</td>
<td>H1N1pdm09</td>
<td>2295 (3)</td>
<td>2213 (2)</td>
<td>Adjusted odds ratio</td>
<td>1.44 (.23–8.90)</td>
<td>. . .</td>
</tr>
<tr>
<td>Pasternak et al [19]</td>
<td>H1N1pdm09</td>
<td>7014 (7)</td>
<td>43663 (131)</td>
<td>Adjusted hazard ratio</td>
<td>1.38 (.22–8.47)</td>
<td>Parity, smoking, maternal age</td>
</tr>
<tr>
<td>Rubinstein et al [24]</td>
<td>H1N1pdm09</td>
<td>7293 (25)</td>
<td>23195 (111)</td>
<td>Relative risk</td>
<td>0.72 (.47–1.11)</td>
<td>. . .</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

* Measure used to calculate pooled (overall) effect estimate.

b Ratio measure not reported in study, calculated using reported number of stillbirth or spontaneous abortion events in vaccinated and unvaccinated groups.

an exposure of H1N1pdm09 vaccination (Figure 2C and Table 2). Two studies reported significant effect estimates of <1 [18, 19], and 4 studies found no association between receipt of the H1N1pdm09 vaccine and stillbirth [20, 22–24] (Table 2).

The association between receipt of 2009 H1N1pdm09 vaccine and spontaneous abortion was null (RR, 0.89 [95% CI, .61–1.29]; Figure 2D). Three studies evaluated this relationship, and all 3 studies found no association between influenza vaccination and spontaneous abortion [19, 20, 22] (Table 3).

1976 Monovalent H1N1(A/NJ/8/76) Vaccination
Only 1 study evaluated the relationship between 1976 monovalent H1N1(A/NJ/8/76) influenza vaccination and stillbirth and found a null result (RR, 2.74 [95% CI, .17–43.5]) [21] (Table 2). This study also examined the association between 1976 monovalent H1N1 influenza immunization and spontaneous abortion and reported a null result (RR, 0.91 [95% CI, .19–4.48]; Table 3) [21].

Bias Assessment and Sensitivity Analyses
Three studies scored a low risk of bias on all 5 domains, 3 studies scored a high or unclear risk of bias on 1 domain, and 1 study scored a high or unclear risk of bias on 3 domains (Table 1). Of the studies with high risk of bias scores, the most common type of bias was selection bias (3 studies), followed by attrition bias (2 studies) and detection bias (1 study). A sensitivity analysis excluding studies that scored a “high” risk of bias on >1 domain showed a more pronounced protective association between vaccination and stillbirth and a similar null association with spontaneous abortion (Supplementary Figure 1). Funnel plots showed little to no publication bias among H1N1pdm09 studies and studies overall (Supplementary Figure 2). An additional sensitivity analysis without restriction on the outcome definitions (in other words, including studies that used alternative outcome definitions that we excluded in our primary analysis) showed the persistence of a protective association with stillbirth [20, 25–29] (Supplementary Figure 3).

DISCUSSION
On the whole, this review provides reassuring evidence for the safety of influenza vaccines in pregnancy with respect to birth...
outcomes of stillbirth, defined as fetal loss after 20 or 22 weeks of gestational age, and spontaneous abortion, defined as fetal loss prior to 20 or 22 weeks of gestational age. Presumably as a result of safety concerns regarding the administration of the 2009 H1N1pdm09 vaccine to pregnant women, most of the studies reviewed focused on the effects of this specific vaccine. It is notable that studies of both adjuvanted and unadjuvanted vaccines were included in our analysis, as adjuvanted vaccines tend to raise more concerns regarding safety. There were an insufficient number of studies focused on seasonal influenza vaccines to make conclusions regarding associations with adverse birth outcomes (there were no studies of trivalent seasonal vaccines that met inclusion criteria).

Therefore, lower rates of stillbirth in women vaccinated during pregnancy could be due to prevention of infection (and thereby prevention of inflammation). However, this finding should be interpreted with substantial caution, as residual confounding of our pooled estimate cannot be ruled out.

There was some variability in the methodological rigor of studies included in the meta-analysis, but almost all studies were of high quality according to the AHRQ bias assessment criteria. The reviewed articles with the most methodologically sound designs were large, population-based retrospective cohort studies that relied on highly accurate and complete information from registries and databases to adjust for potentially confounding factors. Both studies that found significant, protective associations between stillbirth and vaccination were large retrospective cohort studies with >50 000 subjects. The largest, most heavily weighted study in our analysis was one of those that scored most favorably based on the AHRQ criteria, with a low risk of bias in all categories. Overall, there was a low amount of bias detected (a high risk of bias in 1 or no domains) in all studies except for 1. A sensitivity analysis excluding this study showed a similar protective association between vaccination and stillbirth and null association with spontaneous abortion.

There are several limitations of this meta-analysis. First, the exclusion of studies that used alternative definitions for stillbirth

### Table 3. Associations Between Maternal Influenza Vaccination and Spontaneous Abortion

<table>
<thead>
<tr>
<th>Study</th>
<th>Vaccine Type</th>
<th>No. Vaccinated (No. Spontaneous Abortion Events)</th>
<th>No. Unvaccinated (No. Spontaneous Abortion Events)</th>
<th>Measure</th>
<th>Effect (95% CI)</th>
<th>Factors Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chambers et al [22]</td>
<td>H1N1pdm09</td>
<td>184 (7)</td>
<td>116 (9)</td>
<td>Adjusted HR, first trimester vaccination</td>
<td>0.84 (.27–2.64)</td>
<td>Propensity score (race, previous elective termination, infection, autoimmune disease)</td>
</tr>
<tr>
<td>Deinard et al [21]</td>
<td>A/NJ/8/76 H1N1</td>
<td>189 (2)</td>
<td>517 (6)</td>
<td>Relative risk</td>
<td>0.91 (.19–4.48)</td>
<td>Propensity score (maternal age, county of residence, degree of urbanization at place of residence, country of birth, parity, history of fetal death in siblings, selected comorbidities, number of hospital admissions and outpatient contacts in 3 years preceding pregnancy, selected drugs, number of drugs used in 6 mo preceding pregnancy)</td>
</tr>
<tr>
<td>Pasternak et al [19]</td>
<td>H1N1pdm09</td>
<td>2736 (20)</td>
<td>32 672 (1649)</td>
<td>Adjusted HR</td>
<td>1.11 (.71–1.73)</td>
<td>Propensity score (maternal age, county of residence, degree of urbanization at place of residence, country of birth, parity, history of fetal death in siblings, selected comorbidities, number of hospital admissions and outpatient contacts in 3 years preceding pregnancy, selected drugs, number of drugs used in 6 mo preceding pregnancy)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio.

a Measure used to calculate pooled effect estimate.
b Ratio measure not reported in study, calculated using reported number of stillbirth or spontaneous abortion events in vaccinated and unvaccinated groups.
and spontaneous abortion (did not define the cutoff between risk periods at either 20 or 22 weeks) may have biased our summary estimate. However, based on the differing etiologies of these 2 conditions and the fact that women are at risk for these outcomes during different periods in pregnancy, there is questionable relevance of composite outcomes including both spontaneous abortion and stillbirth. Even so, a secondary analysis without restriction on the outcome definitions showed the persistence of a protective association with stillbirth [20, 25–29]. There was a modest number of studies (7) included in this meta-analysis. The statistical methods that were used to describe interstudy heterogeneity and calculate pooled estimates are low-powered in analyses with few studies. However, the tests used were those that were described as most appropriate for small sample sizes, and statistics were not reported for subgroups in which the number of studies was determined to be too small to estimate reliable measures. Lastly, only 1 study of a nonpandemic H1N1 vaccine met our inclusion criteria, and therefore we cannot make any conclusions about the effect of seasonal vaccines on risk of adverse pregnancy outcomes.

Several directions for further research are indicated by these results. Although the H1N1pdm09 vaccine has been extensively studied, more research on the association between trivalent, seasonal influenza vaccination and pregnancy outcomes of stillbirth and spontaneous abortion is needed. If prevention of influenza results in reduced incidence of adverse birth outcomes, it is reasonable that the potential benefit from influenza vaccination varies by season dependent upon the degree of similarity of the vaccine strains to the circulating strains and the resulting level of influenza morbidity. Only 1 study (Pasternak et al) performed sensitivity analyses that adjusted for maternal influenza infection, in an effort to assess the effect of averted influenza infection on fetal outcomes. Multiseason studies of influenza vaccine would also provide further evidence for this theory. Second, not all included studies stratified their analyses by trimester of vaccination to clearly define the risk profiles for immunization at various stages of pregnancy. None of the studies considered the timing of vaccination, which would have been impacted by the point during the influenza season at which the vaccine became available and was recommended for use in pregnant women, relative to the period of maximal influenza circulation. Finally, studies of rare birth outcomes require large sample sizes to provide sufficient power to detect association with an exposure. Although several studies in this analysis were very large, few studies reviewed examined stillbirth or spontaneous abortion as a primary outcome, and power calculations (if performed) were performed for other endpoints. Additional studies must focus primarily on these outcomes to have sufficient power to detect associations involving rare events.

This review provides further evidence of safety of maternal influenza immunization with respect to stillbirth and spontaneous abortion. Further research must continue to investigate these associations to more completely characterize the effect of influenza vaccination during pregnancy on adverse birth outcomes.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


