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Effect of Maternal Influenza Vaccination on Spontaneous Abortion and Stillbirth:

A systematic review and meta-analysis

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Abstract

Effect of Maternal Influenza Vaccination on Spontaneous Abortion and Stillbirth:

A systematic review and meta-analysis

By Kristin Bratton

Influenza vaccination is recommended in pregnancy and has been demonstrated to have beneficial effects on the health of both the mother and infant. Although the effects of antenatal influenza vaccination on other adverse birth outcomes have been characterized, this is the first systematic review meta-analysis of the association between influenza vaccination during pregnancy and stillbirth and spontaneous abortion. We identified and analyzed studies that assessed outcomes of stillbirth or spontaneous abortion after administration of influenza vaccine during pregnancy. A literature search was conducted using the electronic databases Medline, EMBASE, Web of Science, Cumulative Index to Nursing or Allied Health Literature, Scopus, Google Scholar, and the Cochrane Central Register of Controlled Trials. 21 studies met inclusion criteria for the descriptive literature review. Seven of these 21 studies were selected for a quantitative meta-analysis, which was restricted to studies that defined stillbirth as fetal loss prior to 20 or 22 weeks and/or spontaneous abortion as fetal loss after 20 or 22 weeks. Data were extracted from articles using a pre-tested tool and a risk of bias assessment was performed for each study. Pooled estimates for the effects of seasonal influenza vaccine, pH1N1 vaccine, and vaccination overall were calculated for each outcome. The pooled relative risk estimate showed a protective effect of influenza vaccination in pregnancy against stillbirth (RR: 0.65, (0.46, 0.84)), and the effect remained significant when the analysis was restricted to the pH1N1 vaccine (RR: 0.68 (0.54, 0.86)). The pooled effect for spontaneous abortion was not significant (RR: 0.97, (0.46, 1.48)). There was no heterogeneity of effect estimates detected for either outcome (I^2=0%). These analyses add to the evidence base for safety of influenza vaccination in pregnancy. The finding of a protective effect of maternal influenza vaccination on stillbirth warrants inclusion of this outcome in future observational and interventional studies.

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BACKGROUND AND LITERATURE REVIEW

Influenza illness and burden

Influenza is a contagious respiratory illness caused by one of several types of influenza viruses. Influenza is spread through direct contact with infected individuals, by contact with contaminated objects, and by inhalation of aerosolized droplets containing the virus. Symptoms of influenza include fever, cough, sore throat, muscle aches, and fatigue, but severity of disease can vary from mild to very severe[1]. The timing and duration of influenza season varies globally: in the temperate Northern Hemisphere and Southern Hemisphere, influenza circulates primarily in the winter months and incidence most often peaks in February; in the tropics, the season either peaks during the rainy season or exhibits little seasonality at all[2].

Influenza illness represents a substantial contribution to morbidity and mortality each year, both domestically and globally. In the United States, annual deaths from influenza range from approximately 3,300 to 49,000 per year[3], and influenza-related disease can be linked to 200,000 hospitalizations per year[4]. Globally, lower respiratory infections account for 3.46 million deaths and 6.1% of all deaths; in low-income countries the proportion of deaths attributable to influenza is much higher, at 11.3%[5]. It is estimated that influenza causes anywhere from 250-500,000 of these deaths, and causes 3-5 million severe cases per year[5]. Morbidity and mortality fluctuate substantially based on the types of virus circulating in a particular season; for example, in years in which H3N2 is a predominant circulating strain, there can be 2.7 times as many deaths[3]. In high-resource countries, high-risk groups for influenza infection are commonly considered to include persons with underlying health conditions (specifically, chronic illnesses), elderly persons, pregnant women, health care workers and young children[6]. In children, influenza is thought to be the cause of 13% of all acute lower respiratory infections (ALRI) globally (out of approximately

20 million episodes of ALRI each year), and 7% of severe ALRI[7], with a 3-fold and 1.5-fold increase, respectively, in the rates in low-income countries.[8]

Treatment of Influenza

Antiviral drugs can be used to treat influenza. In the United States, there are two such drugs: oseltamivir and zanamavir, both neuraminidase inhibitors with activity against both influenza A and B viruses. Other medications, called M2 ion-channel inhibitors, or adamantanes, are effective against influenza A viruses. However, resistance to these medications has risen in the past several influenza seasons and therefore they are not currently recommended for clinical use[9]. Although antiviral agents can be highly effective in treating influenza, they are too expensive for widespread use in many low-income countries (one treatment course of oseltamivir costs around $15).[10]

Influenza vaccination

Vaccination, however, is the most effective method of protection against influenza. Currently, there are several different types of influenza vaccine in use. Inactivated influenza vaccines (IIVs) are administered intramuscularly and come in both trivalent and quadrivalent formulations. The trivalent IIV protects against three viruses—two influenza A viruses (H3N2 and H1N1) and one influenza B virus; the quadrivalent protects against an additional influenza B virus. Live, attenuated influenza vaccine (LAIV) is administered intranasally and also comes in both a trivalent and quadrivalent form. A relatively new influenza vaccine, recombinant hemagluttinin inactivated vaccine (RIV), is made without egg proteins, antibiotics, or preservatives[11].

The standard, trivalent inactivated influenza vaccine is currently approved for children over 6 months of age. A quadrivalent formulation is available in injectable form, as well as in the form of a nasal spray, which is approved for healthy individuals from 2-49 years of age. Live, attenuated influenza vaccines are can be administered to persons 18 and older. A high-dose TIV

is approved for persons over 65 years of age and RIV is approved for those 18 through 49 years of age.

Although children 6 months of age and younger are one of the highest risk groups for influenza complications, they cannot receive the influenza vaccine and must rely on either maternal antibodies or vaccination of close family members and caregivers for protection against illness. Additionally, vaccination is not recommended in persons who have previously experienced a severe reaction to influenza vaccine, have a previous history of Guillan-Barre syndrome in the 6 weeks following a previous vaccination, or who are moderately ill with fever.

Pregnant women as a risk group

Pregnant women are at increased risk for serious complications from influenza when compared to healthy individuals and as a result are considered a priority group for influenza vaccination by the World Health Organization and the Centers for Disease Control and Prevention[6,12]. In the 1950's, the Advisory Committee on Immunization Practices (ACIP) began to recommend yearly influenza vaccination in pregnant women and this recommendation has been endorsed by The American College of Obstetricians and Gynecologists, The American Academy of Pediatrics, and the American Academy of Family Practitioners[13].

Recent research has supported these recommendations: during the 2009 pH1N1 pandemic, pregnant women were 40.5 times more likely to be hospitalized and to need mechanical ventilation[14], and were at a significantly increased risk of influenza-associated mortality[15]. Data suggest that out of every 10,000 women in their third trimester (without other risk factors) in a standard influenza season of 2.5 months, 25 will be hospitalized with influenza-related morbidity.[16] Late initiation of antiviral treatment in pregnant women further increases the risk of serious influenza-related complications[17].

Influenza infection in pregnant women also has a substantial impact on the health of the fetus. Although vertical transmission of influenza from mother to fetus is very rare, other effects of

influenza infection have been well-documented. Data from the 1918 H1N1 and 1957 H2N2 influenza pandemics suggest an increased risk for pregnancy loss and preterm delivery among women who were infected with the pandemic strain[18-21]. Studies of the most recent H1N1 pandemic demonstrate increases in the rates of perinatal mortality, stillbirth[22], and preterm delivery[23] among women infected with the 2009 H1N1 virus. Seasonal influenza has also been shown to contribute to adverse birth outcomes: research has shown a reduction in mean birth weight of infants among women with influenza-like illness[24] and an increase the proportion of infants born SGA in women hospitalized for respiratory illness during pregnancy[25].

Safety of influenza vaccine

An important consideration for both providers and pregnant patients has been the safety of influenza vaccine administration in pregnancy. Pregnant women are considered a 'vulnerable' population, and therefore few large-scale vaccine safety studies have included sufficient numbers of pregnant women to detect a link between influenza vaccination and rare adverse pregnancy outcomes. Further, because the recommendation for antenatal vaccination has been in place for over 50 years, there are very few prospective, randomized controlled trials studying the effects of the influenza vaccine in pregnant women. Although passive reporting systems (e.g. VAERS and Vaccine Safety Datalink) capture a proportion of adverse events that may or may not be related to vaccine receipt, it is challenging to make conclusions about the relative effects of vaccination comparing vaccinated and unvaccinated individuals based on this data. Observational studies provide some information with respect to this question, but are often complicated by confounding as a result of the 'healthy vaccinee' effect; which arises because women who seek out and receive the influenza vaccine, during pregnancy and otherwise, are typically different based on a series of characteristics from those women who do not.

However, the epidemiological and clinical research that has been done has consistently shown that maternal immunization is a safe and effective intervention to protect against influenza

infection in both the mother and fetus. Several pre-2009 pH1N1 studies have demonstrated the safety of seasonal influenza vaccines[13,26-28] with respect to illness episodes in mothers and birth outcomes of infants. Most recently, the recommendation of the 2009 pH1N1 vaccine for use in pregnant women prompted a series of studies designed to assess the safety and risk of adverse effects associated with receipt of this inactivated influenza vaccine in pregnancy. During the development of the pH1N1 vaccine, several clinical trials[29-31] and observational studies[32-35] included pregnant women and confirmed the safety of antenatal administration[36]. Much of this research contributes to the evidence base not only of the safety of influenza vaccination in pregnancy, but also the myriad additional benefits of maternal vaccination for the mother and the fetus.

Benefits of antenatal immunization

The benefits of antenatal immunization have been shown to extend beyond prevention of influenza in pregnant women receiving the vaccine. Infants of women vaccinated during pregnancy were shown to have a 42% lower risk of influenza-like illness (ILI) requiring hospitalization and a 41% lower risk of laboratory-confirmed influenza up to 6 months of age[37]. Other studies have reported similar results demonstrating the protective effect of influenza vaccination against influenza and ILI on the infant[28,38,39]; however, one study found no association between antenatal immunization and medically-attended respiratory illness in infants[40] (although it was noted that this finding did not eliminate the possibility that vaccination led to a reduction in illness incidence). Overall, the research has shown that passive immunity is transferred from vaccinated pregnant mothers to the fetus and this results in a lower risk of several different influenza outcomes in young infants.

Influenza vaccination has also been demonstrated to have an impact on birth outcomes. During periods of circulating influenza, antenatal immunization has been shown to reduce the proportion of infants born small for gestational age (SGA) by 18.9% and increase mean birth weight by 7%,

suggesting that vaccination has some degree of influence over intrauterine growth of the fetus[41]. Another study showed that during a period of local, regional, and widespread influenza activity, the odds of premature birth among vaccinated women were reduced by 70% as compared to those among unvaccinated women, and a significant protective association was detected between maternal vaccination and reduced likelihood of SGA[42].

Interestingly, these effects have differed based on the socio-demographic characteristics of vaccinated women; protective associations between antenatal immunization and adverse birth outcomes were more pronounced among black women and women with low SES. These groups are also known to be at higher risk than the general population for influenza-related morbidity[43].

Uptake of influenza vaccine during pregnancy

Among pregnant women and in general in the United States, uptake of influenza vaccine peaked during the 2009 H1N1 pandemic and has remained stagnant in the most recent influenza seasons. During the 2012-13 influenza season (October 2012-January 2013), 50.5% of pregnant women reported to have received the influenza vaccine. Recommendation of the vaccine by a health care provider had a substantial impact on receipt: women who were recommended and offered the vaccine by a healthcare provider were most likely to receive the vaccine (70% of these women received the vaccine), and women who were not recommended the vaccine by a provider were much less likely (13% of these women received the vaccine). Women least likely to receive the vaccine were 18-24 year olds, of non-Hispanic Black race, had an education less than a college degree, were unmarried, lived below the poverty level or were infrequent seekers of health care[44]. Attitudes towards the safety and efficacy of the vaccine also impact uptake. Data from the CDC show that coverage among women who had favorable attitudes towards the influenza vaccine was much higher (64.2%) than women who had a negative attitude towards the vaccine (9.8%). 33.2% of women who reported being vaccinated reported that their primary reason for choosing vaccination was to protect their infant from influenza, while 20.5% of women who were not

vaccinated reported that they were concerned about the safety risk to their infant[44]. This data indicates that perceptions of the vaccine play a critical role in determining coverage rates, and that advocacy and community-based efforts that aim to change these attitudes can perhaps have a significant impact on influenza immunization rates in the United States.

INTRODUCTION

Pregnant women are at an especially high risk of influenza-associated morbidity and mortality[16,45-47]. As a result, the inactivated influenza vaccine has been considered an essential element of prenatal care since the 1950s and has been recommended during any trimester of pregnancy since the mid-2000s[48-50]. Benefits of antenatal immunization have recently been demonstrated to extend beyond preventing influenza episodes in mother. A randomized controlled trial in Bangladesh reported a significant reduction in influenza burden in infants of women vaccinated during pregnancy[28], and other studies have reported a lower proportion of infants born premature or small for gestational age (SGA) among women vaccinated against influenza antenatally[41,42,51].

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 U.S. and Canada, misconceptions about the risk of influenza vaccination during pregnancy and concerns about safety of the influenza vaccine are commonly cited reasons for declining vaccination[52,53]. Evidence that antenatal immunization may be protective against adverse birth outcomes may prove important in increasing coverage of influenza vaccine in this high-risk group. Further, synthesis of the evidence surrounding maternal influenza vaccination and adverse pregnancy outcomes is critical to providing high-quality evidence-based prenatal care. The demonstration of

protective or harmful effects, if any, of influenza vaccination on the fetus may inform further recommendations for use of influenza vaccine in pregnant women.

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 small-for-gestational age birth. A putative biological mechanism for the relationship between influenza vaccination and stillbirth or spontaneous abortion may prove altogether different from that of preterm birth and related outcomes, requiring specific study to assess the potential risk factors and effects involved. Thus, systematic reviews of studies that examine the relationship between maternal vaccination and these birth outcomes are critical for confirming the safety of influenza vaccination during pregnancy for the mother and fetus.

This systematic review and meta-analysis is the first to summarize the literature examining the effect of maternal influenza vaccination on birth outcomes of spontaneous abortion (miscarriage) and stillbirth.

METHODS

Study Selection

We sought studies assessing the effect of influenza vaccination during or prior to pregnancy on birth outcomes of spontaneous abortion and stillbirth. Relevant articles considered an exposure of seasonal or pH1N1 influenza vaccination in any stage of pregnancy or immediately prior to conception and measured an outcome of either spontaneous abortion or stillbirth. After the literature search process was complete, we restricted the analysis to studies that defined spontaneous abortion as fetal loss at less than 20 weeks gestational age and stillbirth as fetal loss at greater than 20 weeks, the definitions used by the American College of Obstetricians and

Gynecologists (ACOG). Studies that defined the cutoff at 22 weeks were also included. The quantitative meta-analysis was also restricted to studies that compared birth outcomes in a vaccinated and unvaccinated group (studies that used surveillance data compared to population rates of birth outcomes were ineligible). Studies that assessed outcomes inconsistent with these definitions were eligible for inclusion only in the descriptive literature review.

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 well as immunological, pharmacological, and non-human 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 (using non-MEDLINE databases to reduce duplicate search results), Web of Science, Cumulative Index to Nursing or Allied Health Literature (CINAHL), Scopus, Google Scholar, and Cochrane Central Register of Controlled Trials. Search terms included the 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 do not use MesH terms.

The titles and abstracts of each article were reviewed against inclusion criteria. Articles found in initial searches were used for reference 'harvesting' 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 pre-defined inclusion criteria were included in the final review. No non-English articles meeting the inclusion criteria were found.

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[54] 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[55]. For each included study, information abstracted included study design and research methods, study subject characteristics, and measures of associations and precision. For studies included in the quantitative meta-analysis, there were five domains of bias for which each individual study was evaluated: selection bias, performance bias, attrition bias, reporting bias, 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 pre-specified 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 on a subset of studies by a second reviewer to ensure accuracy of data collection and risk of bias score assignment.

Statistical Analyses

A quantitative meta-analysis was performed using the subset of studies with appropriately defined outcome measures for stillbirth and spontaneous abortion. Forest plots were constructed for overall effect of vaccination and pH1N1 vaccination. Ratio measures and confidence intervals were calculated using OpenEpi[56] for studies that did not calculate ratio measures comparing vaccinated and unvaccinated groups but the necessary information was reported. Hazard ratios and relative risks 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 I^2 and the corresponding p-value, were calculated with the log relative effect measures using a random effects model. Forest plots were used to display effect size data on all studies included in the quantitative analysis and a summary measure was calculated for subgroups in which there were more than one study reporting ratio-based measures using the Dersimonian and Laird method[57]. Funnel plots were constructed to detect the existence of publication bias. Meta-analysis statistical procedures were performed in STATA version 13.1.

RESULTS

Literature search results and qualitative analysis

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 28 remaining records underwent a full text review. Seven more studies were located through manual searching of the cited references from these 28 articles. Of the 35 studies that underwent a full text review, 14 were removed because the exposure or outcome measures did not meet inclusion criteria. 21 studies were included in the descriptive literature

review (Figure 1). Of these, 7 were consistent with our requirements for inclusion in the quantitative meta-analysis.

Of the studies included in the descriptive literature review, retrospective cohort studies made up the largest proportion (n=9), followed by prospective observational cohort studies (n=7), surveillance-based studies (n=3), cross-sectional studies (n=1) and case-control studies (n=1). 17 studies assessed birth outcomes associated with receipt of 2009 pH1N1 vaccine and five studies considered seasonal influenza vaccine exposure. 6 studies were primarily focused on the effect of the vaccine adjuvant. 16 studies assessed outcomes of spontaneous abortion and stillbirth (according to our definitions, although terminology to describe these outcomes varied), four studies assessed an outcome of stillbirth only, and 1 study spontaneous abortion only. 17 studies considered exposure as vaccination in any trimester, two studies during the second and third trimesters, one study during the first trimester only, and one study did not report this information. 17 studies assessed individual outcomes of spontaneous abortion, stillbirth, or both; 4 studies assessed only a composite outcome including both.

Quantitative Meta-Analysis

Of the 7 studies included in the quantitative meta-analysis, 2 were retrospective cohort studies, 4 were prospective cohort studies, and 1 was a cross-sectional study. Six studies assessed outcomes associated with pH1N1 vaccine receipt and 1 study assessed seasonal vaccine receipt. Five studies examined outcomes of both stillbirth and spontaneous abortion (Figure 2, 3). For purposes of analysis, we categorized studies into 3 groups according to vaccine type: (1) all influenza vaccines (7 studies); (2) seasonal influenza vaccine (1 study); and (3) pH1N1 vaccine (6 studies). These categories were not mutually exclusive; for example, a study assessing the effect of a pH1N1 vaccine would fall into first and third groups.

Overall impact of influenza vaccination

Overall, there was a significant protective effect of vaccination against stillbirth (Relative risk: 0.69 (0.54, 0.87)). Of the 7 individual studies that assessed the impact of any type of vaccination on stillbirth, 4 studies found relative effect measures less than 1. The two studies reporting significant relative effect measures were the largest studies. Fell et al. reported an adjusted relative risk of 0.66 (0.47, 0.91) for fetal loss, defined as fetal loss at greater than or at 20 weeks gestation, and Pasternak et al. reported an adjusted hazard ratio of 0.44 (0.20, 0.94) for stillbirth, defined as fetal loss at 22 weeks gestational age or greater. Five studies found that vaccination had no effect on stillbirth rates and reported relative risks of 1.10 (0.47, 2.57)[58], 2.74 (0.17, 43.5)[59], and 0.23 (0.01, 3.93)[60] with stillbirth defined as fetal loss at gestational age greater than 20 weeks, and adjusted odds ratios of 1.44 (0.23, 8.90)[61] , and 0.72 (0.47, 1.11)[62], with stillbirth defined as loss at gestational age greater than 22 weeks. A sensitivity analysis excluding studies with high risk of bias on more than one domain showed a similar effect (Relative risk: 0.68 (0.54, 0.86)). Overall, there was no variation in effect size attributable to heterogeneity (I^2=0%, p for heterogeneity=0.584).

The pooled effect of any vaccination on spontaneous abortion was nonsignificant (Relative risk: 0.99, (0.68, 1.43)). Of the 4 studies that assessed the impact of any type of vaccination on spontaneous abortion, all found null effects. These studies reported risk ratios of 0.60 (0.22, 1.63)[58], 0.91 (0.19, 4.48)[59] and an adjusted HR of 0.92 (0.31, 2.72)[63], with spontaneous abortion defined as fetal loss prior to 20 weeks gestational age, and an adjusted hazard ratio of 1.11 (0.71, 1.73)[64], with spontaneous abortion defined as fetal loss prior from 7-22 weeks gestational age. An I^2 test showed no significant heterogeneity of effect measures among these studies (I^2=0%, p for heterogeneity=0.742).

Vaccination against pH1N1

The pooled effect of pH1N1 vaccination on stillbirth was a relative risk of 0.68 (0.54, 0.86). Six out of the 7 studies that assessed the effect of maternal influenza vaccination on stillbirth examined an exposure of pH1N1 vaccination. Thus, the results are similar to those for any type of vaccination. 2 studies reported effect estimates less than 1[64,65] and 4 studies found no association between receipt of the 2009 pH1N1 vaccine and stillbirth[58,63,61,62]. An I^2 test for studies of pH1N1 vaccination showed no significant heterogeneity of effect measures (I^2=0.0% p for heterogeneity: 0.590).

The effect of pH1N1 vaccine on spontaneous abortion was null (Relative risk: 0.99 (0.68, 1.45). Four studies evaluated the effect of 2009 pH1N1 maternal influenza vaccination on spontaneous abortion. All four studies found no association between influenza vaccination and spontaneous abortion[58,61,63,64]. There was no significant inter-study heterogeneity among studies (I^2=0%, p for heterogeneity=0.539).

Vaccination against seasonal influenza

Only one study evaluated the relationship between seasonal influenza vaccination and stillbirth and found a null result (Relative risk: 2.74 (0.17, 43.5))[59]. This study also examined the effect of seasonal influenza immunization on spontaneous abortion and reported a null result (Relative risk: 2.74 (0.17, 4.48))[59].

Bias assessment

Risk of bias was assessed for each study. Three studies were found to have a low risk of bias on all domains, three studies were found to have one domain for which risk of bias was high or unclear, and one study scored a high or unclear risk of bias on three domains. Of the studies with high bias scores, the most common type of bias was selection bias (three studies), followed by

attrition bias (two studies), and detection bias (one study) (Figure 3). Funnel plots to detect publication bias showed little to no publication bias among pH1N1 studies and studies overall (Figure 5).

DISCUSSION

On the whole, this review provides reassuring evidence for the safety of influenza vaccines in pregnancy with respect to birth outcomes of stillbirth and spontaneous abortion. Published studies suggest a protective effect of influenza vaccination during pregnancy with respect to stillbirth, defined as fetal loss after 20 or 22 weeks gestational age, and a null effect of antenatal influenza vaccination on spontaneous abortion, defined as fetal loss prior to 20 or 22 weeks gestational age. Presumably as a result of safety concerns regarding the administration of the 2009 pH1N1 vaccine to pregnant women, most of the studies reviewed focused on the effects of this specific vaccine. There were an insufficient number of studies focused on seasonal influenza vaccines to make conclusions about their effects on adverse birth outcomes.

A putative mechanism for the protective effect of influenza vaccination on stillbirth may be through prevention of the inflammation associated with influenza infection. Influenza infection during pregnancy has been associated with poor birth outcomes, including preterm birth and low birth weight,[47] and research has shown that this effect is at least partially mediated through a pathway involving inflammation.[66] Although vaccination itself induces an inflammatory response, this response is milder and more transient as compared to that of natural influenza infection. Although the details of the relationship between vaccination during pregnancy and birth outcomes are not expressly clear, this finding should motivate further study to describe the basis for an ostensible protective effect.

Research shows that infection contributes to a large proportion of stillbirths: a review estimated that 10-25% of stillbirths in high-income countries can be attributed to infection (this proportion is likely as high 50% in low and middle-come countries)[67]. Influenza infection has been shown to increase stillbirth rates significantly, although the magnitude of effects reported have varied[20,22,68]. Further, previous research suggests stillbirth rates display seasonality, with a recent systematic review citing several studies that show higher stillbirth rates in the winter months[69]. The reason for this seasonality is yet unclear.

A putative mechanism for the protective effect of influenza vaccination on stillbirth may be through prevention of the inflammation associated with influenza infection. Influenza infection during pregnancy has been associated with poor birth outcomes, including preterm birth and low birth weight,[47] and research has shown that this effect is at least partially mediated through a pathway involving inflammation.[66] Although vaccination itself induces an inflammatory response, this response is milder and more transient as compared to that of natural influenza infection. Although the details of the relationship between vaccination during pregnancy and birth outcomes are not expressly clear, this finding should motivate further study to describe the basis for an ostensible protective effect.

In previous studies examining the effect of maternal influenza immunization on preterm birth and small-for-gestational age birth, protective effects were most pronounced in periods of widespread influenza virus circulation[42]. The 2009-2010 influenza season saw relatively high transmission and morbidity[70] and featured a vaccine that was well-matched to the circulating H1N1 virus strain, resulting in a higher number of prevented cases of influenza as compared to typical influenza seasons. The majority of studies in this review focused on the 2009 pH1N1 vaccine and because these studies are likely reflective of situations in which vaccination was truly protective against infection and subsequent inflammation, this strengthens the causal inference

that can be made between vaccination and protection against stillbirth. However, this also suggests that the magnitude of protective effect of influenza vaccination may vary by season, contingent upon the prevalence of influenza infection and the level of protection conferred by the vaccine. This effect is likely to be much lower than our reported pooled estimates in a typical influenza season.

There was some variability in the methodological rigor of studies included in the meta-analysis but almost all studies were of high quality according the AHRQ criteria. Reviewed articles with the most methodologically sound designs included large, population-based retrospective cohort studies that relied on highly accurate and complete information from registries and databases. Both studies that found significant, protective associations between stillbirth and vaccination were large (greater than 50,000 subjects) retrospective cohort studies. The largest, most heavily weighted study was one of the studies with the least amount of bias based on the AHRQ criteria; this study was one of two that showed a significantly protective effect of influenza vaccination on stillbirth. Overall, there was a low amount of bias detected (a risk of bias in one or no domains) in all studies except for one. A sensitivity analysis excluding studies with a high risk of bias on more than one domain showed a more pronounced protective effect of vaccination on stillbirth and a similar null effect on spontaneous abortion (Figure 6). Importantly, although many influenza studies must address the potential for "healthy user" bias, in which healthier individuals are more likely to be vaccinated, studies of this population involve women of reproductive age who are relatively healthy and therefore this is unlikely to be of concern.

There are a few limitations of this meta-analysis. First, the exclusion of studies that used alternative definitions for stillbirth and spontaneous abortion (did not define the cutoff between risk periods at either 20 or 22 weeks) may have biased our summary effect estimate. However, based on the differing etiologies of these two 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. The effect measures of studies that used alternative definitions were, in our view, not comparable to those of the included studies. Moreover, a secondary analysis without restriction on the outcome definitions (in other words, including studies in the descriptive literature review that reported any kind of relative risk measure) showed the persistence of a protective effect against stillbirth (Figure 7). Second, there are several established tools designed to determine the extent of bias in individual studies and it is yet unclear which tool is optimal, particularly for observational studies. However, the AHRQ tool used was well suited to the types of studies included in the review (primarily prospective and retrospective cohort studies)[55]. Third, bias assessment by the reviewers was potentially subjective, particularly in the absence of reporting of methods. Corroboration of bias assessment results by two reviewers eliminated some of this uncertainty. Lastly, the statistical methods that were used to describe inter-study heterogeneity and calculate pooled estimates are low-powered in situations 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.

Several directions for further research are indicated by these results. Although the pH1N1 vaccine has been extensively studied, more research on the effect of seasonal influenza vaccination on pregnancy outcomes of stillbirth and spontaneous abortion is needed. Assuming that 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 strain to the circulating strain. Multi-season studies of influenza vaccine would provide further evidence for this theory. Further, studies must stratify analyses by trimester of vaccination in order to clearly define the risk profiles for immunization at various stages of pregnancy. Lastly, studies of rare outcomes, including stillbirth and spontaneous

abortion, require large sample sizes to provide sufficient power to demonstrate association with an exposure. Several studies in this analysis were very large, notably the registry-based retrospective cohort studies. However, few studies reviewed examined stillbirth or spontaneous abortion as a primary outcome, thus power calculations (if performed) were performed for other study outcomes. Further studies must focus primarily on assessing these outcomes in order to have sufficient power to detect associations involving such rare events.

This review provides further evidence of safety of maternal influenza immunization. Our analysis suggests that antenatal influenza vaccination may confer a protective effect against stillbirth, particularly during seasons of high influenza circulation and in which the influenza vaccine is well-matched to the circulating influenza strain. However, we did not find an effect on risk of spontaneous abortion. Further research must confirm these associations using studies that aim to elucidate the biological mechanism of the protective effect of influenza vaccination and have adequate power to detect rare birth outcomes.

Public Health Implications

This analysis reinforces the importance of vaccinating pregnant women during periods of influenza circulation, particularly in seasons of pandemic influenza. Demonstration that influenza immunization during pregnancy may prevent a fraction of preterm, small-for-gestational age birth, and stillbirth greatly strengthens the evidence base for influenza vaccination during pregnancy. Although more research is needed to confirm our conclusions and to more extensively characterize the impacts of vaccination during different influenza seasons, this finding may have substantial implications for public health policy and practice.

Maternal influenza vaccination has already been proven highly cost-effective in both seasonal influenza periods and in influenza pandemics.[71] The suggestion that maternal influenza vaccination can reduce stillbirth by as much as 30% during pandemic influenza seasons can likely improve cost-effectiveness estimates of influenza vaccination domestically and globally. Pandemic preparedness often involves maximizing use of scarce resources and making key, informed decisions about which preventive measures should be implemented (and to what extent) in order to most effectively protect the public health. The finding that influenza vaccine not only prevents influenza but also reduces the risk of adverse birth outcomes in pregnant women adds to the health benefits of influenza vaccination, and should accordingly impact policy-making on a national and global level.

Vaccine acceptance is a key component of maximizing the benefits of maternal influenza vaccination during periods of both seasonal and pandemic influenza. In high-resource countries, in which influenza vaccine supply is adequate but demand is often low, effective communication of the various benefits of maternal influenza vaccination may serve to increase vaccine uptake among pregnant women. This is supported by evidence that shows vaccine receipt is closely linked to women's perceptions of the vaccine safety: in a 2013 CDC report, 20.5% of women in the United States who were not vaccinated reported that they were concerned about the safety risk to their infant.[44] Although there are a complex series of factors that contribute to vaccine acceptance and uptake, we are hopeful that through strengthening the evidence for the safety and benefits of vaccination during pregnancy, this finding may play a role in influencing influenza vaccine uptake among pregnant women in the future.

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Figure 1: Flow Diagram of Included and Excluded Studies

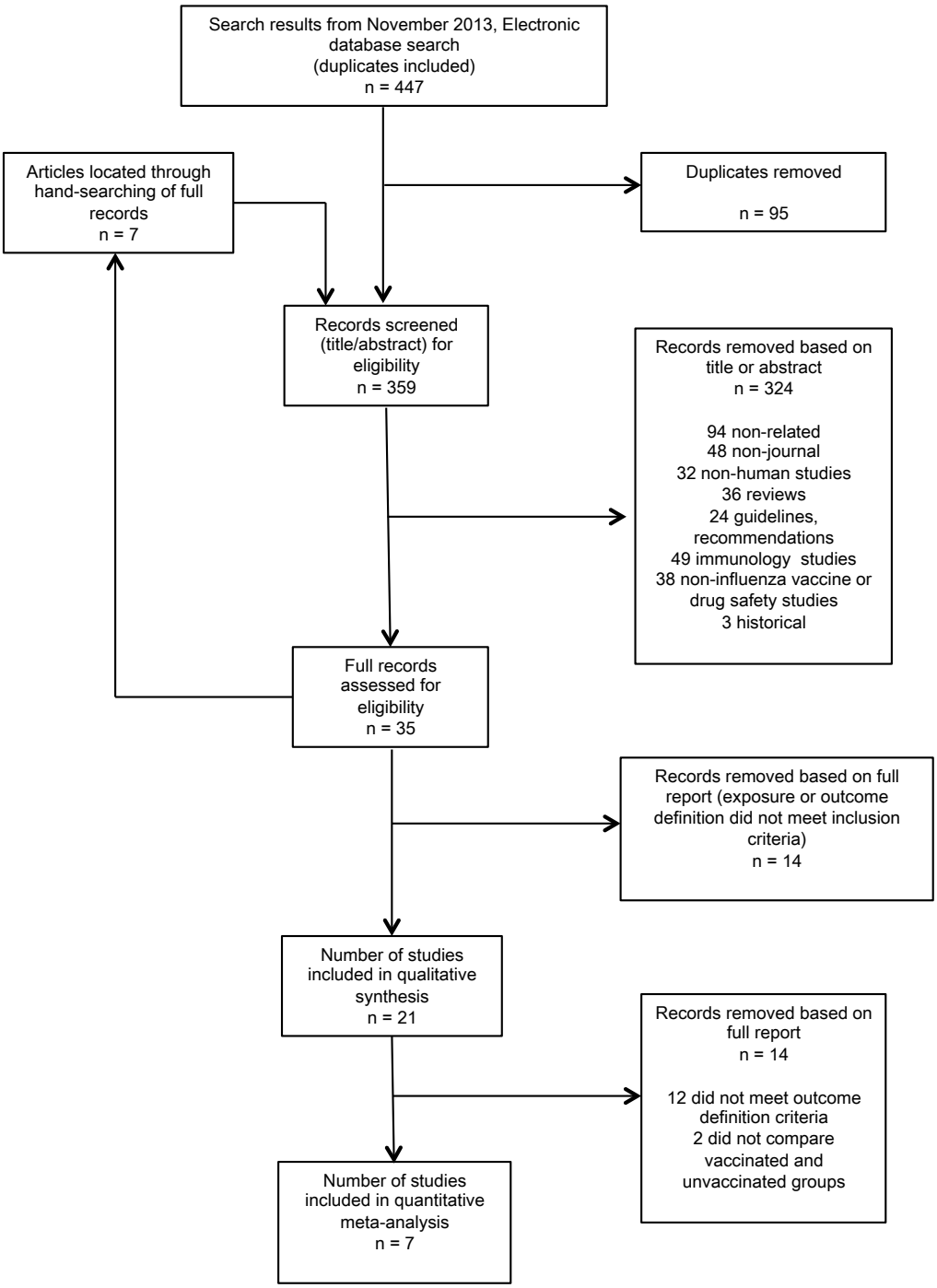


Figure 2: Characteristics of eligible studies for descriptive literature review

Author, year published (location of study)	N	Setting	Study design	Intervention	Vaccination trimester	Stillbirth (SB) definition	Spontaneous abortion (SAB) deifnition
Cantu et al., 2013 (USA)	3104	maternity clinics	Prospective cohort	2009 H1N1 vaccine	NR	≥20 weeks	<20 weeks
Chambers et al., 2013 (USA, Canada)	1032	phone-based consultation service	Prospective cohort	2009 H1N1 vaccine	All	NR (assumed >20 weeks)	<20 weeks
Chavant et al., 2013 (France)	2415	Vaccination centers, maternity departments	Prospective cohort	2009 H1N1 vaccine	All	>500g or >22 weeks	<500g or <22 weeks
Deinard et al., 1981 (USA)	815	Obstetric clinics	Prospective cohort	InfA/NJ /76 vaccine	All	≥20 weeks	<20 weeks
Fell et al., 2012 (Canada)	55570	Birth registry	Retrospective cohort	2009 H1N1 vaccine	All	≥20 weeks	--
Goldman et al., 2013 (USA)	241	Passive surveillance and phone interviews	Retrospective cohort	2009 H1N1 vaccine and 2009 seasonal vaccine	All	NR	NR
Haberg et al., 2013 (Norway)	113331	registry/ surveillance	Retrospective cohort	2009 H1N1 vaccine	Second/ Third	>12weeks	
Heikkinen et al., 2012 (Netherlands, Italy, Argentina)	4508	Hospitals, midwife practices, GP offices	Prospective cohort	2009 MF59-adjuvanted H1N1 vaccine	All	≥22weeks	<22weeks
Huang et al., 2011 (Taiwan)	14474	passive surveillance	Surveillance	Adjuvanted 2009 H1N1 vaccine	All	≥20 weeks	<20weeks
Irving et al., 2013 (USA)	486	VSDS health care organizations	Matched case-control	2005 seasonal vaccine	First	>5 weeks	--
Kallen et al., 2012 (Sweden)	238824	Birth registry	Retrospective cohort	2009 H1N1 vaccine	All	--	NR
Lin et al., 2012 (Taiwan)	396	Hospitals	Retrospective cohort	Adimflu-S vaccine	All	NR	
Moro et al., 2011 (USA)	148	Passive surveillance	Surveillance	TIV or LAIV	All	≥20 weeks	<20 weeks
Opperman et al., 2012 (Germany)	1652	Phone consultation service	Prospective cohort	2009 H1N1 vaccine	All	NR	NR
Pasternak et al., 2012 (Denmark)	54585	Database	Retrospective cohort	2009 H1N1 vaccine	All	≥22weeks	7-22 weeks
Rubinstein et al., 2013 (Argentina)	30000	Hospitals	Cross-sectional	2009 /H1N1 MF59-adjuvanted vaccine	All	>22 weeks	--
Sammon et al., 2012 (UK)	39863		Retrospective cohort	2009 H1N1 vaccine	All	≥8 weeks	
Sheffield et al., 2012 (USA)	85783	Hospital	Retrospective cohort	2003-2008 seasonal vaccine	All	≥500g	--
Tavares et al., 2011 (UK)	267		Prospective cohort	AS03-adjuvanted H1N1 2009 vaccine	All	≥24 weeks	<24 weeks
Tsai et al., 2010 (USA)	103	clinical trial database	Surveillance	Adjuvanted vaccine	All	Abnormal pregnancy outcome, including ectopic pregnancy, SAB, SB	

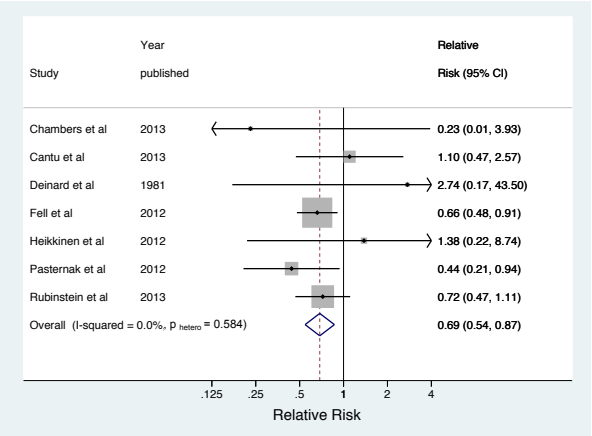
Figure 3: Effect measures for stillbirth and spontaneous abortion among studies included in quantitative meta-analysis

Study	Stillbirth Measure	Stillbirth Effect (95% CI)	Spontaneous abortion Measure	Spontaneous abortion Effect (95%CI)	Bias Risk Assessment
All influenza vaccines					
Chambers et al	Risk ratio, first trimester	0.57 (0.03, 9.57)	Adjusted HR, first trimester vaccination	0.84 (0.27, 2.64)	
	Risk ratio, any trimester	0.23 (0.01, 3.93) [b]	Adjusted HR, <20weeks gest.age vaccination	0.92 (0.31, 2.72) [b]	Risk of selection bias
Cantu et al	Risk ratio	1.10 (0.47, 2.57) [a,b]	Risk Ratio	0.60 (0.22, 1.63) [a,b]	Risk of selection bias
Deinard et al	Risk ratio	2.74 (0.17, 43.5) [a,b]	Risk ratio	0.91 (0.19, 4.48) [a,b]	Risk of selection bias Risk of attrition bias Unclear risk of detection bias
Fell et al	Adjusted risk ratio	0.66 (0.47, 0.91) [b]			Low risk in all categories
Heikkinen et al	Adjusted odds ratio	1.44 (0.23, 8.90)			
	Adjusted hazard ratio	1.38 (0.22, 8.47) [b]			Low risk in all categories
Pasternak et al	Adjusted hazard ratio	0.44 (0.20, 0.94) [b]	Adjusted hazard ratio	1.11 (0.71, 1.73) [b]	Unclear risk of attrition bias
Rubinstein et al	Risk ratio	0.72 (0.47, 1.11) [a,b]			Low risk in all categories
Seasonal vaccine					
Deinard et al	Risk ratio	2.74 (0.17, 43.5) [a,b]	Risk ratio	2.74 (0.17, 4.48) [a,b]	
H1N1 vaccine					
Cantu et al	Risk ratio	1.10 (0.47, 2.57) [a,b]	Risk Ratio	0.60 (0.22, 1.63) [a,b]	
Chambers et al	Risk ratio, first trimester	0.57 (0.03, 9.57)	Adjusted risk ratio, first trimester	0.57 (0.03, 9.57)	
	Risk ratio, any trimester	0.23 (0.01, 3.93) [b]	Adjusted risk ratio, any trimester	0.23 (0.01, 3.93) [b]	
Fell et al	Adjusted risk ratio	0.66 (0.47, 0.91) [b]			
Heikkinen et al	Adjusted odds ratio	1.44 (0.23, 8.90)			
	Adjusted hazard ratio	1.38 (0.22, 8.47) [b]			
Pasternak et al	Adjusted hazard ratio	0.44 (0.20, 0.94) [b]	Adjusted hazard ratio	1.11 (0.71, 1.73) [b]	
Rubinstein et al	Risk ratio	0.72 (0.47, 1.11) [a,b]			

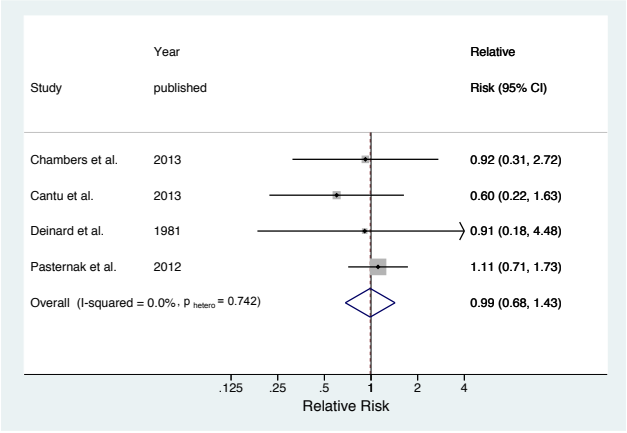
[a] Ratio measure not reported in study, calculated using reported cell counts,[b] Measure used to calculate pooled effect estimate

Figure 4: Forest Plots for stillbirth and spontaneous abortion, overall and H1N1 only

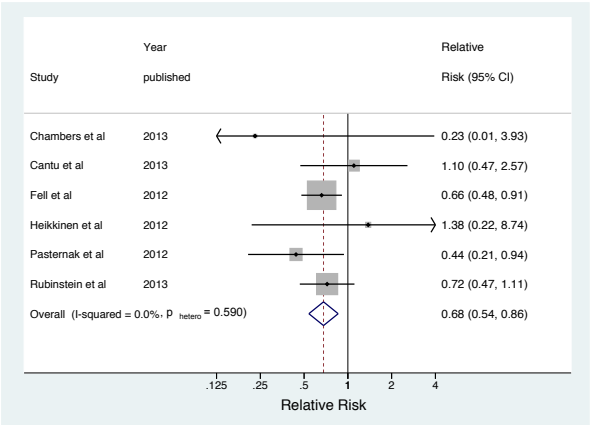
a. Stillbirth, overall



b. Spontaneous abortion, overall



c. Stillbirth, H1N1 only



d. Spontaneous abortion, H1N1 only

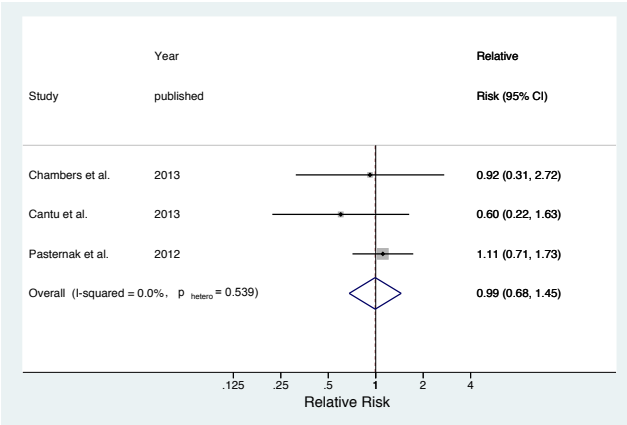
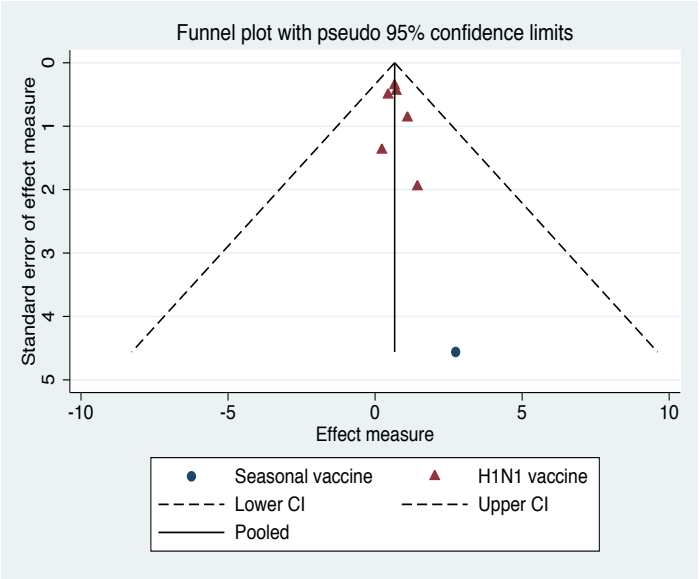


Figure 4: The center of each box represents the point estimate of effect reported for each study and arrows represent confidence interval limits outside of the shown range. The size of the grey shaded box corresponds to the weight of the study in the meta-analysis, (larger studies weighted more heavily). The dotted red line and blue diamond represent the pooled point estimate and corresponding confidence interval.

Figure 5: Funnel Plots for stillbirth and spontaneous abortion

a. Stillbirth



b. Spontaneous abortion

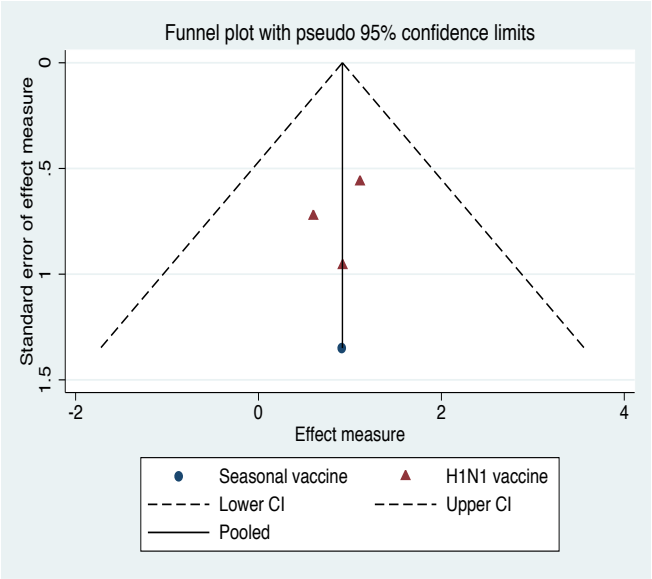
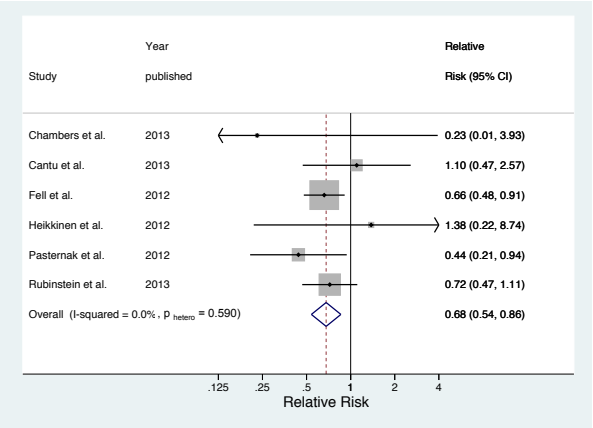
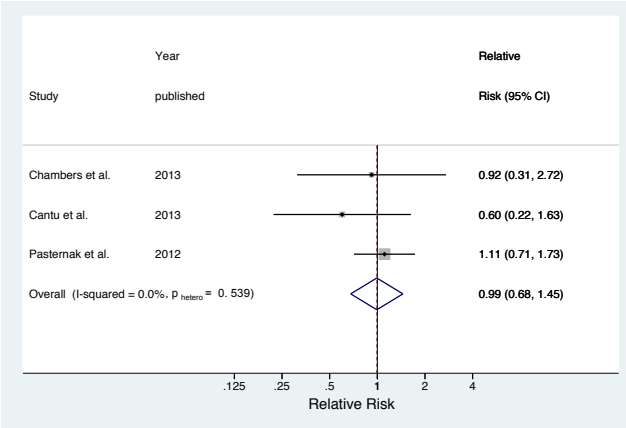


Figure 6: Sensitivity Analysis, studies with lowest risk of bias

a. Stillbirth, overall



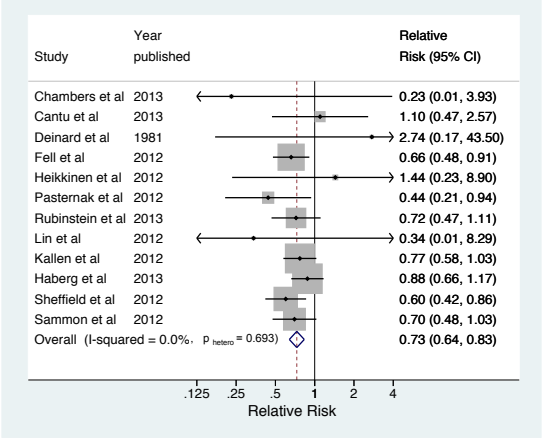
b. Spontaneous abortion, overall



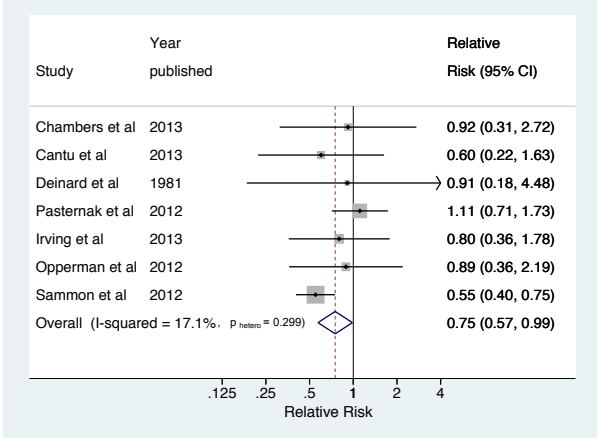
Supplementary Figure 2: The excluded study was the only one that examined seasonal influenza vaccine; all studies above are pH1N1 studies (the overall and pH1N1-only forest plots are identical). The center of each box represents the point estimate of effect reported for each study and arrows represent confidence interval limits outside of the shown range. The size of the grey shaded box corresponds to the weight of the study in the meta-analysis, (larger studies weighted more heavily). The dotted red line and blue diamond represent the pooled point estimate and corresponding confidence interval.

Figure 7: Forest plot analysis without restrictions on outcome periods

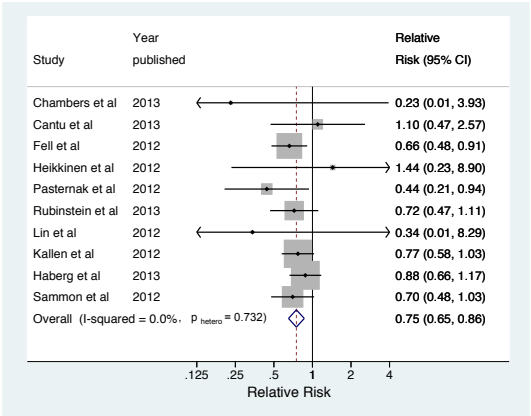
a. Stillbirth, overall



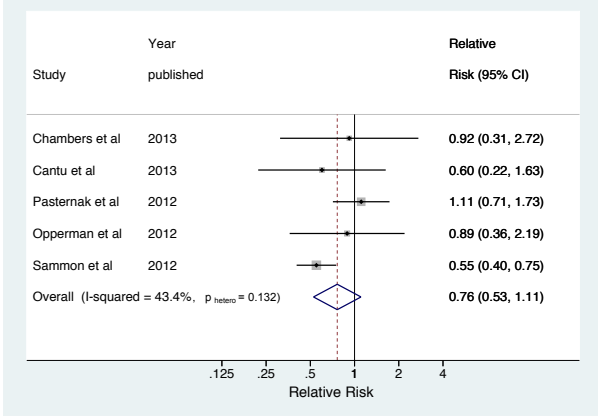
b. Spontaneous abortion, overall



c. Stillbirth, H1N1 only



d. Spontaneous abortion, H1N1 only



Supplementary Figure 3: The center of each box represents the point estimate of effect reported for each study and arrows represent confidence interval limits outside of the shown range. The size of the grey shaded box corresponds to the weight of the study in the meta-analysis, (larger studies weighted more heavily). The dotted red line and blue diamond represent the pooled point estimate and corresponding confidence interval.