Influenza vaccination during pregnancy: a systematic review of effectiveness and safety.

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Abstract

Background

Pregnant women are the World Health Organisation's top priority group for influenza vaccination and it is the primary intervention to protect pregnant women, their foetus, and infant from influenza infection. However, it is considered to be an expensive public health measure and data on the effectiveness and safety of the vaccine has been lacking and inconsistent. Evidence of the vaccine's effectiveness and safety is critical to the decision making process of governments and policy-makers, as well as clinicians and pregnant women.

Objectives

To synthesise the best available evidence on the effectiveness and safety of influenza vaccination during pregnancy for pregnant women, their foetus, and infant up to six months of age.

Inclusion criteria.

Types of participants

Pregnant women with or without risk factors for complications from influenza infection, their foetus, and infants up to the age of 6 months.

Types of intervention

Inactivated influenza vaccination administered to pregnant women of any trimester.

Types of studies

Studies using quantitative research methods were considered for this systematic review.

Types of outcomes

This systematic review considered studies that reported on the effectiveness of maternal influenza vaccination at reducing the rate and severity of influenza and influenza-like illness for pregnant women and infants up to six months of age. The review also investigated the safety outcomes for pregnant women and foetus following influenza vaccination during pregnancy including adverse events, spontaneous abortion, foetal death, premature birth, low birth weight, small for gestational age, and congenital malformation.

Search strategy

An extensive search of the literature was undertaken to find both published and unpublished English language studies between the inception of each database to April 2013.

Methodological quality

Papers selected for retrieval were assessed by two independent reviewers for methodological validity prior to inclusion in the review.

Data collection

Data were extracted from included papers using data extraction tools.

Data synthesis

Data were, where possible, pooled in statistical meta-analysis. Where statistical pooling was not possible the findings were presented in narrative and table form.

Results

A total of 39 relevant studies were included in the review following critical appraisal. Studies investigating birthing and foetal outcomes were reported in 28 studies. Adverse event outcomes for pregnant women were present in 24 studies. The effectiveness of maternal influenza vaccination in reducing illness in pregnant women and infants up to 6 months was reported in 13 studies.

Conclusions

Influenza vaccine administered during pregnancy is effective and provides a similar reduction in influenza-like illness as it does for a healthy adult population. Despite this, there is no evidence on the effectiveness of the influenza vaccine at reducing severe illness or hospitalisation in pregnant women. Infants of pregnant women vaccinated during their second or third trimester can expect to have reduced rates of influenza, and influenza related hospitalisation, for their first 6 months of life.

Influenza vaccination during pregnancy had no association with adverse outcomes for the foetus including premature birth, small for gestational age infants, congenital malformation, spontaneous abortion, and foetal death.

Declaration

I, Mark McMillan, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide, and where applicable, any partner institution responsible for the joint-award of this degree.

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Mark McMillan

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Chapter 1. Introduction

Chapter 1 provides the context and methodological basis of the systematic review. The chapter also outlines the researcher's background and importance of this review.

1.1 Context of the review

1.1.1 Description of the condition for pregnant women

Influenza virus circulates worldwide, usually during winter in temperate climates. In most cases it causes an acute self-limiting respiratory infection and febrile illness and can sometimes be accompanied with other symptoms such as myalgia, headache, malaise, and fatigue. However, complications from influenza can be serious and there is an increased risk of harm to pregnant women, particularly in the last two trimesters of pregnancy.[1-5]

The increase in risk to pregnant women is thought to be due to the immunologic and physiologic changes that occur during pregnancy. The immune system in pregnant women changes so that they can tolerate a genetically foreign foetus, with half of the genes from the father. These changes are not well understood and are thought to result in a shift from cell-mediated immunity towards humeral immunity.[6] When combined with physiological changes such as increased heart rate, oxygen consumption, and decreased lung capacity, pregnant women become more susceptible to infectious diseases, including influenza.[6] As the pregnancy progresses and the physiologic changes are greater, the risk of hospital admission with a respiratory illness also increases.[3, 5]

Influenza related mortality appears to be rare in otherwise healthy pregnant women.[5] Not one influenza related death was reported in two studies conducted in the USA that spanned a combined period of 20 influenza seasons.[2, 7] However, the influenza virus has the potential for a sudden antigenic shift that can result in a more pathogenic subtype resulting in pandemic spread. The novel influenza virus places people at greater risk due to little or no immunity to the virus.[8] During pandemic periods pregnant women have been at an increased risk of respiratory complications, as well as hospital and intensive care admissions.[9-13] The only reliable epidemiological evidence of increased influenza related mortality prior to the 2009 influenza pandemic was associated with the 1918 (H1N1) influenza pandemic and 1957 (H2N2) influenza pandemic.[5]

Although there appears to be very low risk of mortality during non-pandemic influenza seasons, there is an increased risk of severe disease and hospitalisation.[14] A 2007 Canadian study by Cox et al. during a non-pandemic period reported that the number of pregnant women admitted to hospital with a respiratory illness increased per trimester of pregnancy, with a rate ratio of 1.7 (95% CI: 1.0 to 2.8) in

the first trimester, 2.1 (95% CI: 1.3 to 3.3) in the second trimester and 5.1 (95% CI: 3.6 to 7.3) in the third trimester.[3] The authors estimated that 68 healthy pregnant women in their third trimester were excess hospital admissions per 100000 persons.[3] This rate was lower than that of healthy children under 2 years of age, but equivalent to rates of American adults aged 15-44 years with a comorbid condition; both of these population groups are recommended to receive the influenza vaccine.[3] Pregnant women who have a coexisting illness or risk factor such as asthma or obesity have an even greater risk of harm from influenza infection.[3, 5, 14] This increase in risk compared to healthy pregnant women was well illustrated by Cox et al., who estimated that women with a comorbidity in their third trimester had 1210 hospital admissions per 100000, compared to the figure of 68 per 100000 for healthy pregnant women.[3]

1.1.2 Description of the condition during the 2009 influenza A (H1N1) pandemic

In April 2009 a novel influenza A (H1N1) virus was identified.[8, 15] The virus spread rapidly around the world and was declared a pandemic on June 11 2009.[15] Early in the pandemic pregnant women were identified as a high-risk group for severe complications from the virus.[15] Most of these early studies were small case series reports.[15] In 2011 a systematic literature review of 120 papers reporting on 3110 pregnant women from 29 countries during the 2009 influenza A pandemic was published.[15] It concluded that pregnancy was associated with increased risk of hospitalisation and intensive care admission, and death. The included studies consisted of 1625 (52.3%) pregnant women who were hospitalised, 378 (23.3%) who were admitted to intensive care, and 130 (8%) who died. The authors of the review reported that pregnant women were disproportionately represented amongst hospitalisations, intensive care admissions, and death.[15] In Australia and the USA, pregnant women made up 6.3% of hospitalisations, 5.9% of ICU admissions, and 5.7% of deaths, whilst only making up 1% of the total population.[15] This was not replicated across all studies and some research has reported lower rates of morbidity and mortality than the studies from Australia and the USA.[15, 16]

Consistent with studies conducted during seasonal influenza seasons, pregnant women with additional risk factors were at an increased risk of severe disease during the 2009 influenza pandemic period.[15] 30.3% of the pregnant women with severe disease had at least one additional risk factor with asthma being the most common, followed by diabetes mellitus, and obesity.[5, 15]

1.1.3 Description of the condition for the foetus

The effect on the foetus in mothers infected with influenza is not as clear. A comprehensive literature review conducted in 2009 reported that there was no consistent association between influenza and adverse pregnancy outcomes.[5] The 2009 review by Skowronski et al. reported that no association has been found between pregnant women who have contracted influenza and pre-term delivery, low birth

weight, low Apgar scores, and delivery complications.[5] A retrospective cohort study by McNeil et al. (2011) included data over a 13 year period and reported that pregnant women who had been hospitalised for respiratory illness during the influenza season were more likely to deliver small for gestational age (SGA) infants, adjusted RR 1.66 (95% CI: 1.11 to 2.49). This finding was not replicated by an earlier study by Hartert et al. (2003).[1] Overall the risk of transplacental transmission is thought to be low, despite case reports of in-utero infection confirmed with viral culture.[10] A 2005 study reported an increased prevalence of congenital malformations in infants of mothers who had an influenza illness during pregnancy, although they concluded that this was unlikely to be a teratogenic effect and could be explained mainly by fever.[11] There is currently some debate about a possible link between influenza contracted in early to mid-trimester pregnant women and schizophrenia.[5] In 2004 a case control study of 64 people with schizophrenia spectrum disorders and 125 controls showed an odds ratio of 7.0 (95% CI: 0.7 to 75.3) between prenatal exposure to influenza during the first trimester and risk of schizophrenia.[17] According to Skowrinski et al. there has more recently been a theoretical link proposed between activation of a maternal immune response to influenza antigens and schizophrenia using animal models.[5] These possible associations need to be further investigated.

In times of pandemic the risk of harm to the foetus of pregnant women rises.[18-20] A retrospective cohort study during the influenza A (H1N1) 2009 pandemic found pregnant women infected with influenza had an increased risk of preterm delivery and perinatal mortality when compared to non-infected pregnant women.[19] This finding is consistent with observations during the pandemic of 1918-20, where women with influenza complicated by pneumonia had increased rates of spontaneous abortion.[20] A prospective cohort study by Pierce et al. (2011) was conducted in the United Kingdom (UK) and reported that pregnant women hospitalised with pandemic influenza also had an increased rate of stillbirth (27 per 1000 total births vs. 6 per 1000 total births; p=0.001), and premature birth adjusted OR 4.0, (95% CI: 2.7 to 5.9).[19] Hårberg et al. (2013) conducted an analysis comparing women who were pregnant outside the pandemic window with those who were exposed to the pandemic, and reported an increased risk of foetal death, adjusted HR 1.26 (95% CI: 1.02 to 1.55). The risk of foetal death increased among women with a clinical diagnosis of influenza, adjusted HR 1.91 (95% CI: 1.07 to 3.41).[18] Even with this evidence, maternal infection with influenza virus is not generally recognised as a risk to foetal survival in the absence of hospitalised maternal illness.[18]

1.1.4 Description of the condition for infants under 6 months of age

Infants under 6 months of age have an immature immune system and are susceptible to complications from influenza. A report from the Centre of Disease Control (CDC) in the USA estimated that children under 5 months of age without high risk conditions had a rate of 1038 hospitalisations per 100,000 between 1973 to 1993.[21] The rates were estimated in years of low immunisation rates.[1] Surveillance

undertaken during the 2003-04 influenza season in the USA by the CDC reported that 18 infants under the age of six months died due to complications from influenza, resulting in the highest mortality rate in children under 17 years of age.[22] Similar to pregnant women, infants aged 0-6 months are more susceptible to complications from influenza if they have an underlying comorbidity such as cardiac, respiratory, neurological, or neuromuscular conditions.[2, 22] During the same period of 1973 to 1993 it was estimated that the number of hospitalisations for children 0-11 months with an underlying comorbidity was 1900 per 100,000.[21]

1.1.5 Evolution of the influenza virus

Influenza is an enveloped, single stranded, negative-sense RNA virus and forms part of the *Orthomyxoviridae* virus family.[23, 24] There are three types of influenza virus core proteins A, B and C, with Influenza A and B the most common causes of human epidemics and thus the most common types produced in vaccines. [23, 25, 26] On the surface of influenza viruses are embedded two antigenic glycoproteins, namely hemagglutinin (HA) and neuraminidase (NA) that induce an antibody response in humans.[26] Each of these has subtypes, with influenza A having three major HA subtypes (H1,H2, and H3) and two major NA subtypes (N1 and N2).[26, 27] These components are also used to describe the strain of the influenza virus e.g., influenza A (H1N1).

Influenza viruses are continually mutating with minor mutations due to 'antigenic drift'. This type of mutation is usually associated with influenza epidemics.[25] Sudden mutations that result in a novel strain are described as being caused by an 'antigenic shift'. These rapid changes result from a pandemic strain, such as occurred in the 2009 influenza season and results in widespread infection and mortality, especially if there is no pre-existing immunity in the human population. These rapid changes are often caused by reassortment between animal and human influenza viruses.[27]

Pandemics due to the influenza virus have occurred throughout history. The most notable was recorded in 1918 to 1919. This was known as the "Spanish Flu" and was estimated to have resulted in 50 to 100 million deaths.[28] [25] Following the 1918 pandemic other notable pandemics have occurred in 1957, 1968, 1977 and 2009.[27]

1.1.6 Description of the influenza vaccine

Humans' immune systems have the ability to remember foreign invaders including the influenza virus. During a 'natural' infection, memory B and T cells are produced that can provide protection against future attacks, however the infection can have serious consequences with severe complications and even death.[29] Vaccines were developed as a safe way of triggering the production of memory B and T cells without the potential risks of contracting the disease.[29] Vaccines have been extremely effective in reducing contagious disease. One example of the potential effectiveness of vaccines is the polio

vaccine. Contracting polio can result in irreversible paralysis for 1 in 200 children under 5 years of age and death in 5% to 10% of those paralysed. Rates of polio since the introduction of a global immunisation program have decreased by over 99% since 1988 from 350000 to 223 cases reported in 2012.[30]

Influenza vaccination is the primary preventive measure to reduce complications from influenza. Unlike many of the other vaccines, regular mutation of the influenza virus from antigenic drift means the vaccine may need to be modified on a yearly basis, or more frequently in the case of a sudden antigenic shift that results in a pandemic strain. Currently trivalent influenza vaccines are recommended to provide protection against three different strains of influenza that are anticipated to be circulating in the northern and southern hemisphere influenza seasons. Inactivated influenza vaccines are currently the most common type of vaccines available and there are three types contained in this review. The first are whole-virion vaccines developed in the 1940s and contain killed or inactivated whole influenza viruses.[31] The second are split-virion vaccines developed in the 1960s in response to the reactogenicity of the whole-virion vaccines, especially in infants and children. These are manufactured by disrupting the whole-virion vaccine with detergents.[31] The third are subunit vaccines that contain surface antigens only.[31, 32] The most common method of administration is parenteral, although live attenuated aerosol and inactivated aerosol are used in some parts of the world.[32] Due to the potential risk to the foetus from live vaccines, inactivated influenza vaccines are the only type recommended for use in pregnant women.

The types of adjuvant added to improve the immunogenicity of vaccines also varies depending on what region or company developed the vaccine. Adjuvants can aid in antigen delivery or target specific immune pathways that improve the immune response to the vaccine.[33] Recently the influenza A (H1N1) 2009 vaccine was manufactured with adjuvants, and the types varied between manufacturers. Licensed adjuvants include aluminium salt and squalene oil-in-water emulsion systems MF59 and ASO3. Whilst adjuvants may improve the immunogenicity of vaccines and appear to be well tolerated, they are another factor that needs to be considered when looking at the safety of specific vaccines.[33]

In 1947 The World Health Organisation (WHO) established a worldwide surveillance system identifying and tracking circulating viral influenza strains. This tracking is used to predict the most likely strains to be circulating in the upcoming influenza seasons around the world and inform the make-up of the vaccine. The accuracy of the match of the vaccine strains to the circulating viral strains has shown to be important in the effectiveness of the influenza vaccine.[34] During the influenza A (H1N1) 2009 pandemic, a monovalent vaccine was manufactured and administered to target the dominant circulating pandemic influenza A (H1N1) virus. Whilst the monovalent vaccine was manufactured using similar methods to the seasonal vaccine, the single strain in the vaccine and increased pathogenicity of the circulating virus warranted a sub group analysis of the research findings in this review.

Infants under 6 months of age are currently unable to be vaccinated. The vaccine is not licensed in this age group as they are likely to have a modest immune response to influenza vaccines.[35] Reduced immunity and effectiveness of vaccines for children under 6 months is also a concern with other potentially serious childhood diseases, and there is a recent focus on vaccination during pregnancy to protect infants from other vaccine preventable diseases.[36]

For infants under the age of 6 months it has been shown that influenza antibodies are transferred from their mother and this may provide some protection for the first months of life.[37] Placental transfer of maternal immunoglobulin G (IgG) can provide short-term passive immunity for the newborn infant.[36] IgG antibodies' unique ability to pass from the mother's blood through the placenta to the foetus, can provide protection for several months until the infant starts producing its own antibodies.[29] The transfer of IgG can start occurring from about 13 weeks gestation, although the majority of IgG transferred occurs in the last 4 weeks of pregnancy,[36] and may be important in regards to the timing of the influenza vaccine during pregnancy. Higher concentrations of IgG in the infant are associated with a longer duration of protection, which in turns means that they will have a shorter period where they may be vulnerable before their immune system matures.[36] There is some research that has raised issues about an infant with a higher level of maternally derived antibodies not having as effective immune response when vaccinated for the first time, and further research is needed in this area to establish its impact on infant vaccination schedules.[36]

Maternal immunoglobulin A (IgA) is present in colostrum and breast milk and also assists in protecting infants.[36] IgA is the main antibody class that protects the mucosal lining of the respiratory tract and digestive system.[29] Influenza A IgA antibodies in breast milk have been shown to be significantly higher in women vaccinated during pregnancy at birth, 6 weeks, and 6 months but not 12 months.[38]

1.1.7 Uptake of the influenza vaccine in pregnant women

The uptake of Influenza vaccination during pregnancy is suboptimal.[39] In the USA the the Advisory Committee on Immunisation Practices (ACIP) and American College of Obstetricians and Gynaecologists recommend that pregnant women have the vaccine. Despite this, in 2004 only 12% of pregnant women were vaccinated. Following the 2009 influenza pandemic during the 2010 to 2011 influenza season 47% of pregnant women were vaccinated.[40] In the 2012-2013 influenza season 50.5% of pregnant women were vaccinated with the influenza vaccine. These levels have improved markedly post the 2009 pandemic, but are still well short of efforts to reach an 80% set in the USA.[39]

The CDC in the USA conducted an Internet based survey in April 2013 with 2047 women between 18-49 years of age who were pregnant during the time the vaccine was available.[40] In this survey they found that women who were non-Hispanic black, had no college degree, were not married, had no

health insurance, were unemployed, lived below the poverty level, had no chronic conditions, and had fewer than six health care provider visits were less likely to get vaccinated.[40] Pregnant women were less likely to get vaccinated if they had a negative attitude to the safety of the vaccine (13% versus 65.6%), or negative attitude to the efficacy of the vaccine (9.8% versus 64.2%).[40]

A total of 72.3% of the women reported having the vaccine recommended to them by a health-care provider and those that received a recommendation were more likely to be vaccinated.[40] The top three reasons women cited as to why they chose to get the vaccine were to protect the infant (33.2%), to protect themselves (20%), and because it was recommended to them by their health-care provider. The top three reasons that women cite as to why they did not have the vaccine were concerns about harming the infant (20.5%), that the vaccine would give them influenza (13.6%), and that the vaccine was not effective in preventing influenza (10.6%).

A qualitative study based on a health belief model using a naturalistic paradigm was conducted in the USA in 2012.[39] The authors used a health belief model to attempt to explain and predict health behaviours, and this was achieved by focusing on the attitudes and beliefs of individuals.[39] Sixty pregnant women were interviewed face to face with 31 who had the vaccine and 29 who rejected the vaccine.[39] Five main themes emerged on the influence for action to be vaccinated, and these were:[39]

- Two-for-one benefit is an important piece of knowledge that influences future vaccination.
- Fear if I do get vaccinated.
- Fear if I don't get vaccinated.
- Women who verbalise that they have 'no need' for the vaccine also fear the vaccine.
- A convenient location reduces a barrier to getting vaccinated.

In Australia vaccine uptake during pregnancy is low with estimates reported by Wiley et al. as ranging between 7% and 40%, although the author does say these findings are based on small samples in specific regions.[41] The Australian Institute of Health and Welfare estimates that there was a 22.8% vaccination uptake of the influenza (H1N1) vaccine in pregnant women during the 2009 influenza season.[42] A survey conducted in 2011 of 815 pregnant women in New South Wales found that 27% of their sample had had the influenza vaccine and that women who were recommended to have the vaccine from a health-care provider were more likely to be vaccinated with an adjusted odds ratio 20.0 (95% CI: 10.9 to 36.9).[41] Concern about the safety of the vaccine was negatively associated with pregnant women being vaccinated with an odds ratio of 0.5 (95% CI: 0.2 to 0.9).[41] The authors concluded that the recommendation from a health-care provider was strongly associated with pregnant women overcoming concerns about the safety of the vaccine.[41] A similar retrospective survey conducted in Western Australia found that 25% of women in their sample who gave birth in 2012

received the influenza vaccine during their most recent pregnancy.[43] The findings again identified barriers in regards to women's beliefs about the safety and effectiveness of the influenza vaccination, with 23% of women saying that the vaccine was unsafe for them and 27% believed it was unsafe for the baby.[43] When asked about effectiveness, 30% thought that it would not protect them from influenza and 26% thought it did not offer protection for the baby.[43]

Seasonal influenza uptake in the UK for pregnant women as a percentage of those registered as seeing a GP was 27.4% in 2011/12 season and 40.3% in 2012/13.[44] In Europe, vaccination rates with the 2009 (H1N1) varied from country to country. A study by Luteijn et al. (2011) tabled the available data from Europe and indicates vaccination rates of the 2009 pandemic (H1N1) vaccine varied between 0% to 54%. Figures from Denmark and Norway have been added from papers included in this review, and also contain births from the 2010 influenza season (table 1).[45]

Table 1 Pandemic 2009 (H1N1) influenza vaccination coverage of pregnant women; available data on EUROCAT per European country.[45]

Country (births 2009)	(H1N1) vaccination rates
Austria (76033)	0 to 24%. Based on personal estimation
Croatia (44794)	20%. Data from Croatian Institute of Public Health, Epidemiology unit
Denmark (53 432)†	13.1% [46]
Finland (60187)	51.2% of mothers giving birth October-December 2009 were vaccinated with Pandemrix according to the Finnish medical birth register.
France (823925)	25 to 49%. Based on CoFluPreg study (maternity wards in Paris)
Germany (664219)	0 to 24%. Based on registry and the Robert Koch Institute
Ireland (73870)	National Summary of Pandemic Influenza Vaccination estimates a vaccination rate of 32.5%
Malta (4136)	0 to 24%. Based on national vaccination register data
Norway (117347)†	54% of pregnant women during their second or third trimester of pregnancy.[18]
Portugal (99896)	0 to 24%. Based on national registry.
Spain (494944)	0 to 24%. Based on personal estimation.

† Includes 2010 season.

The uptake of influenza vaccination during pregnancy throughout the world is varied and some countries are well short of the proportion of the pregnant population they would consider optimal to be vaccinated.

1.1.8 Current recommendations, strategies and policies for influenza vaccination

Worldwide many health authorities including the WHO recommend pregnant women receive an influenza vaccination during any trimester of pregnancy.[47] In 2012 the WHO Strategic Advisory Group

for Experts on immunisation recommended pregnant women *"as the most important risk group for inactivated seasonal influenza vaccination"*(p.207).[48] That shift in priority resulted in a revision of the 2005 position paper on influenza vaccines, and was based on the evidence *"of substantial risk of severe disease in this group"*(p.207).[48] Additionally, considerations for targeting this group included *"operational feasibility and the opportunity to prioritise and strengthen maternal immunisation programs."*(p.207)[48] The WHO have a stance that recommends each country; "*(i) establish its burden of disease for influenza, (ii) introduce a control policy, (iii) implement vaccination, managing supply and demand and cost, and (iv) establish targets and measure outcomes."(p.4)*[48]

The practice of vaccination of pregnant women is not new and has been performed since the 1950s. ACIP in the USA officially recommended the vaccine for all pregnant women in 1997.[49] Originally, this recommendation was for vaccination in the second and third trimester only, but was changed in 2004 to include any trimester.[50] Other countries have gradually followed suit with the ACIP recommendations, some hastened by the influenza A (H1N1) 2009 pandemic. Countries such as Australia and the UK recommend and fund influenza vaccine programs for groups considered at higher risk, which includes pregnant women of any trimester. Other countries such as USA and Canada recommend influenza vaccine from six months of age for all people.[49, 51]

In June 2012 the first Asia-Pacific influenza summit was held in Bangkok with the objectives of reviewing the state of official influenza control policies in Asia-Pacific countries. The summit was specifically interested in identifying and communicating successfully increased influenza vaccine uptake in the region, as well as developing policy and advocacy approaches to increase vaccine uptake, especially for high-risk groups.[48] The group called Asia-Pacific Alliance for the Control of Influenza (APACI) consists of members from nine countries, namely Australia, China, Hong Kong, India, Indonesia, Korea, New Zealand, Philippines, and Thailand.[48] The summit noted that there are multiple influenza vaccine policies throughout the region with varying adherence to the WHO recommendations, and some of the countries did not have any policy about vaccinating high risk groups, including pregnant women.[48] They also identified that communicating the risk of influenza to policy makers, healthcare professionals and patients is an area that the region wished to improve, especially in respect to having appropriate messages to different cultural groups.[48]

Variability of influenza policy around the world is not dissimilar to that noted in the Asia-Pacific region. Luteijn et al. (2011) collated strategies used throughout Europe and these are outlined in table 2.[45] One of the clear differences around the world was the recommendation of which trimester pregnant women should be vaccinated.

Table 2 Pandemic vaccination policy overview by country for pregnant women and the general population (only those with a policy included)[45]

Country	Trimester			Vaccination strategy for general population
	1	2	3	
Austria		X	X	The entire population was offered the vaccine and certain groups were prioritised
Belgium		X	X	Priority groups only
Denmark		X	X	Priority groups only
Finland	X	X	X	The entire population was offered the vaccine and certain groups were prioritised
France		X	X	The entire population was offered the vaccine and certain groups were prioritised
Germany		X	X	The entire population was offered the vaccine and certain groups were prioritised
Greece		X	X	The entire population was offered the vaccine and certain groups were prioritised
Hungary	X	X	X	The entire population was offered the vaccine and certain groups were prioritised
Ireland		X	X	The entire population was offered the vaccine and certain groups were prioritised
Italy		X	X	The entire population was offered the vaccine and certain groups were prioritised
Malta	X	X	X	The entire population was offered the vaccine and certain groups were prioritised
Netherlands		X	X	Priority groups only
Norway		X	X	The entire population was offered the vaccine and certain groups were prioritised
Portugal		X	X	Priority groups only
Slovakia	X	X	X	The entire population was offered the vaccine and certain groups were prioritised
Slovenia	X	X	X	The entire population was offered the vaccine and certain groups were prioritised
Spain	X	X	X	Priority groups only
Sweden	X	X	X	The entire population was offered the vaccine and certain groups were prioritised
Switzerland		X	X	The entire population was offered the vaccine and certain groups were prioritised
UK	X	X	X	Priority groups only

1.1.9 Influenza vaccination during the first trimester of pregnancy

The developing foetus is at most risk during the first trimester,[52] so the timing of the vaccination in relation to the date of conception is an important factor to consider. As described in section 1.1.8, there were differing recommendations in countries around the world with the rollout of the pandemic influenza vaccine in 2009/10. Medications are rarely tested for teratogenicity in controlled clinical trials,[52] and the influenza vaccine is no different.[37, 53-59] Evidence for safety is often derived from epidemiological studies, individual case reporting, and animal studies. This can cause some issues with the quality of evidence available.[52] All treatment carries some risk. The risk of harm to pregnant women from contracting influenza increases as the pregnancy progresses to the second and especially the third trimester. The risk of harm to pregnant women from complications of contracting influenza during their first trimester is only marginally more than the healthy non-pregnant population.[5] These factors need to be considered when collating the evidence and making recommendations regarding influenza vaccination during pregnancy.

1.1.10 Influenza vaccination outcome measures

Influenza is seasonal in temperate climates and there is normally an epidemic period during winter when people are at risk of contracting the virus.[32, 60] Investigating the effectiveness of influenza vaccines requires timing the study period to coincide with the influenza season and ideally the peak period of virus circulation. This is achieved by various methods in influenza research and includes using the

winter period as a proxy for the influenza season, which is sometimes referred to as the putative season. Other more accurate methods include identification of the first and last influenza virus circulating in the community, or setting a threshold of the amount of influenza cases, or respiratory illness that needs to be occurring in the community.[32] The antigenic match of the vaccine with the circulating strain is also important when assessing the effectiveness of the vaccine. Vaccines that are well matched with the circulating strain have been shown to be more effective than poorly matching vaccines.[32] To measure the rates of influenza a set of symptoms is often used to identify a case of influenza. During peak periods of influenza circulation an acute febrile respiratory illness is often diagnosed as influenza without laboratory testing, as this can be a reasonable predictor of laboratory confirmed influenza cases and a prudent use of resources.[60] However, there are circumstances where it is clinically important to establish the diagnosis via laboratory testing and is often performed in hospitalised patients with influenza-like symptoms.[60] The case definitions can vary between studies, regions, and age groups, although definitions commonly require a sudden onset of symptoms, fever and at least one respiratory symptom.[61]

Study outcomes are often defined or identified by the use of International Classification of Disease (ICD) categories, such as the 9th revision (ICD-9) or the 10th revision (ICD-10). The WHO and 10 international collaborating centres work at standardising and classifying conditions so they are able to be compared worldwide.[62] In 1976 the British Paediatric Association created specific coding for congenital abnormalities[62] that is now used internationally with extensions in use for both ICD- 9 or ICD-10 classifications.[62-64] This type of coding is also commonly used in retrospective studies that are using electronic databases to identify conditions of interest such as influenza-like illness, comorbidities and birthing outcomes.

Safety outcomes during clinical trials are commonly separated into two main categories, namely 'serious adverse events' and 'adverse events'. Serious adverse events are considered an adverse event that results in death, is life threatening, requires hospitalisation, results in significant on-going incapacity, or causes a congenital abnormality or birth defect.[65] Adverse events are defined as unfavourable changes in health that occur during the trial or specified period following the trial.[65] In some studies investigating adverse events for pregnant women, 'adverse events of special interest' were reported that included conditions of specific interest to the researchers. To evaluate the safety of the vaccine for the foetus outcome measures such as spontaneous abortion, foetal death, premature birth, SGA infant, and low birth weight infants are sometimes used. Definitions for some of these outcomes vary between regions and studies. Spontaneous abortion or miscarriage is defined as clinically recognised pregnancy loss before the 20th week of gestation, although there are other accepted definitions including the definition used by the WHO that defines it as expulsion of an embryo or foetus weighing less than 500g.[66] Foetal

death or stillbirth usually describes foetal death later than the 20-week period or more than 500g.[67] There are other alternatives to these definitions and some are used in the papers included in this review. Preterm birth is consistently defined as delivery that occurs prior to 37 weeks gestation and very preterm as prior to 32 weeks gestation. Low birth weight is defined as less than 2500g and very low birth weight less than 1500g.[68]

1.2 Importance of this review

Protecting pregnant women and infants from influenza is a priority, as seen during the 2009 influenza A (H1N1) pandemic, where pregnant women developed severe complications from influenza infection. Maternal immunisation is considered the optimal means to provide protection to both mother and infant for an expanding number of vaccines. In addition to influenza, pertussis vaccine has now been approved for use in the UK, USA and Australia. Safety and effectiveness data are required to ensure confidence in the implementation of these programs and ensure effectiveness of these expensive public health interventions. Doubts about the effectiveness and safety of the vaccine still heavily influence whether pregnant women decide to have the vaccine, and it requires weighing the evidence of benefit against the risk of harm. Therefore evidence of the vaccine's effectiveness and safety is critical to the decision making process by government and policy-makers, as well as clinicians and pregnant women. No recent systematic reviews were found that contained studies of the influenza A 2009 pandemic vaccine following a search of the JBI Library of Systematic Reviews, Cochrane Library, Pubmed, Cinahl and Prospero.

A large amount of new evidence is now available following the pandemic influenza A (H1N1) 2009 outbreak. This systematic review assesses all available studies in an attempt to provide clarity on the effectiveness and safety of influenza vaccination in pregnant women.

1.3 Researchers experience in this field

I have worked as a Registered Nurse since completing my training in 1992 at The Queen Elizabeth Hospital in Adelaide. I moved to Darwin in 1995 and worked in the Royal Darwin Hospital Infectious Disease unit and also in Infection Control. In 2008 I was a Research Officer at Menzies School of Health Research involved in a year-long prospective epidemiological study on sepsis in the Top End, and research on sepsis-associated micro vascular dysfunction, as well as a prospective study investigating bacteraemic *Acinetobacter* Pneumonia in Tropical Australia. I also worked for the Centre of Disease Control in Darwin during the 2009 (H1N1) influenza pandemic, and coordinated fever clinics for healthcare workers and the general public. During that period I participated in the rollout and administration of the influenza (H1N1) 2009 vaccine, which included pregnant women.

1.4 Methodological basis of review approach

Evidence based healthcare is a relatively new concept and was considered to become an organised movement in the early 1990's.[69] The start of the Evidence-based healthcare movement was influenced by the growth in laboratory research, the growth in clinical research, and the realisation that despite the increased scientific knowledge healthcare practice was not uniformly being influenced by the findings.[69] Evidence-based medicine, as it was originally called, evolved through the 1990's from a medically focused model on literature effectively guiding practice, to a "*bottom-up approach that integrates the best external evidence with individual clinical expertise and patient choice"* as described by Sackett et al in 1998.[70]

The Joanna Briggs Institute (JBI) was part of the origins of the organised evidence-based healthcare movement through the 1990's. In 2005 Pearson et al. published a conceptual model of evidence-based healthcare.[71] The model contained four major cyclical components derived from the understandings in the field of evidence-based healthcare. The four components of the JBI evidence-based healthcare process model are:[71]

1. Healthcare evidence generation.
2. Evidence synthesis.
3. Evidence (knowledge) transfer.
4. Evidence utilisation.

Healthcare evidence generation is considered a complex concept that means different things to different people.[71] The JBI model seeks to be inclusive of a wide range of activities and interventions, as well as the type of evidence required to substantiate their worth.[71] In the JBI model the 'healthcare evidence generation' is inclusive of evidence that pertains to the feasibility, appropriateness, meaningfulness, and effectiveness of an activity or intervention.[71]

This comprehensive quantitative systematic review question and approach is designed to synthesise the evidence on the effectiveness and adverse events of influenza vaccine during pregnancy. This approach is limited to evidence derived from clinical empirical research. Pearson et al. define 'effectiveness' as *"the extent to which an intervention, when used appropriately, achieves the intended effect. Clinical effectiveness is about the relationship between an intervention and clinical or health outcomes."(p.210)*[71] These relationships between interventions and health outcomes are encompassed in the questions that are pursued in this review.

In the JBI model 'evidence synthesis' there are key components that are considered to be fundamental to the synthesis of evidence.[70] The cornerstone of evidence synthesis is the systematic review of a condition, intervention or issue.[71, 72] Pearson et al. (2005) outline the key steps that are required to be

undertaken to conduct a systematic review and they are the basis of the methodology prescribed by JBI and followed in this review.[73] The key steps include:[71]

1. Development of a rigorous protocol.
2. Stating the question that will be pursued in the review.
3. Identifying the criteria that will be used to select the literature.
4. Detailing the strategy that was used to identify all relevant literature within a specified timeframe.
5. Assess and critically appraise each study based on quality considerations.
6. Detail how data will be extracted.
7. Setting out a plan of how the data will be synthesised.

Systematic reviews are further defined by Tufanaru et al (2012) as *"a research synthesis study that identifies relevant studies, appraises their methodological quality, and summarises their results using a transparent and explicit scientific methodology."* [74] This definition touches on some of the strengths of a systematic review over traditional literature reviews, namely synthesis of studies, appraisal of quality, transparency, identification of all relevant studies, and use of scientific methodology.

This systematic review on effectiveness and safety is just one piece of the puzzle regarding influenza vaccination during pregnancy. There are likely to be other factors at play that are affecting the uptake of the influenza vaccine during pregnancy which require different methodology to complement the evidence obtained from this review. The basis behind the methodology used in this review is that gaps in empirical evidence exist, as no recent systematic review investigating the effectiveness or safety of the vaccine has been published, and a large body of new evidence post the 2009 influenza pandemic is now available.

Chapter 2. Systematic review protocol

Chapter 2 outlines the systematic review protocol and includes the types of participants, interventions, studies, and outcomes included in this systematic review. This chapter also describes the methods used for searching, critical appraisal, data extraction, and data synthesis. The protocol and methods of analysis for this review were specified in advance and published in the JBI Database of Systematic Reviews and Implementation Reports and PROSPERO International prospective register of systematic reviews.[75, 76]

2.1 Review question(s) and objective

The overall objective of the review was to provide clarity on the effectiveness and safety of influenza vaccination in pregnant women. The systematic review set out to find, appraise, extract, and synthesise the available evidence on the effectiveness and safety of influenza vaccination for mother, foetus, and infant up to 6 months of age.

More specifically the systematic review questions were:

1. What are the beneficial effects of influenza vaccination during pregnancy for pregnant women, their foetus, and infant up to six months of age?

2. What are the adverse effects of influenza vaccination during pregnancy for pregnant women, their foetus, and infant up to six months of age?

2.2 Criteria for considering studies

2.2.1 Types of participants

This review focused on pregnant women with or without risk factors for complications from influenza infection, their foetus, and infants up to the age of 6 months.

2.2.2 Types of intervention(s)/phenomena of interest

- Inactivated whole-virion, split-virion, or subunit influenza vaccine administration, irrespective of antigenic configuration, or adjuvant, administered via any route, any dose, to pregnant women of any trimester.
- Studies investigating the monovalent influenza A (H1N1) 2009 vaccine were assessed separately, as well as in combination with the standard trivalent vaccine.

Comparator: Pregnant women not vaccinated against influenza

2.2.3 Types of studies

This review considered both experimental and epidemiological study designs including randomised controlled trials, non-randomised controlled trials, quasi-experimental, before and after studies, prospective and retrospective cohort studies, case control studies, and analytical cross sectional studies.

2.2.4 Types of outcomes

- Effectiveness of maternal influenza vaccination at reducing the rate and severity of influenza and influenza-like episodes for pregnant women and infants up to six months of age.
- Adverse events for pregnant women following influenza vaccination including, but not limited to, local reaction, fever, anaphylaxis, Guillain-Barré Syndrome, and maternal death.
- Protective and adverse effects of maternal influenza vaccination on the foetus including, but not limited to, spontaneous abortion, stillbirth, premature birth, birth weight, foetal growth, and congenital malformation.

The case definition of influenza was accepted as a clinical diagnosis of respiratory and systemic symptoms as defined by the author, and/or laboratory confirmed influenza using viral isolation and/or serology.

Severity for the mother and infant was assessed by hospitalisation and/or death, and/or severe disease requiring intensive care admission.

2.3 Review methods

2.3.1 Search Strategy

The search strategy aimed to find both published and unpublished studies. A three-step search strategy was utilised in this review. An initial limited search of PubMed and Embase was undertaken followed by analysis of the text words contained in the title and abstract, and of the index terms used to describe the article. A second search using all identified keywords and index terms was then undertaken across all included databases. Thirdly, the reference list of all identified reports and articles was searched for additional studies. Only studies published in English were considered for inclusion in this review. All articles from the inception of the database to April 2013 were considered for inclusion. A search for unpublished studies was conducted using key words and restricted to populations and study designs of interest. Search strategy details are further described in Appendix I.

The databases searched included:

PubMed, Embase, Scopus, and Web of Science.

The search for unpublished studies included:

Scirus, MedNar, ProQuest Dissertation and Theses, Australian Digital Thesis program.

Appendix I provides the search statements and grids of all databases searched.

2.3.2 Assessment of methodological quality

Papers selected for retrieval were assessed by two independent reviewers for methodological validity prior to inclusion in the review using standardised critical appraisal instruments from the JBI Meta-analysis of Statistics Assessment and Review Instrument JBI-MAStARI (Appendix II). Disagreements that arose between the reviewers were resolved through discussion. Studies were excluded if they did not meet at least 5 of the criteria in the critical appraisal instruments.

2.3.3 Data extraction

Data were extracted from papers included in the review using the standardised data extraction tool from JBI-MAStARI (Appendix III).

2.3.4 Data synthesis

Quantitative data were, where possible, pooled in statistical meta-analysis using Cochrane Review Manager (Revman) version 5.2.5. Effects estimates and generic inverse variance meta-analysis were used as results consisted of adjusted odds ratios following regression analysis of large unmatched cohorts. DerSimonian and Laird random-effects model was used due to the potential clinical variability. The random effects model estimate provides a more conservative estimate of overall effect size. Statistical heterogeneity was assumed at probability-value (p) less than 0.05 using the Chi-square test for homogeneity, as well as the I-squared (I^2) statistic and Tau-squared (Tau^2) greater than 1. Where statistical pooling was not possible the findings were presented in narrative form including tables to aid in data presentation. Numbers needed to vaccinate were calculated using GraphPad Software.

Chapter 3. Results

Chapter 3 contains the results of the systematic review. Firstly it describes the studies included and their methodological quality as a whole. The results for each outcome are then described in detail along with tabling of individual results and meta-analysis where appropriate.

3.1 Description of studies

From the initial database search 5338 articles were identified. After removal of duplicates and evaluation of title and abstract, 5265 records were excluded. Full text articles were retrieved for 79 records, with 35 excluded. Of the studies excluded, 18 were due to pregnant women making up a small sub-population of a larger cohort and not being analysed separately, or not identified in the cohort. Of the remainder, 11 were excluded because the studies contained no outcome of interest for this review, four were reports on surveillance, one was a summary of data in other papers, and one was available in abstract only. One study had 6.2% of its participants vaccinated in the four weeks prior to conception that could not be separated from outcome results of the women vaccinated during pregnancy. This study was retained, as the preconception group was a small number and they were specifically investigating potential adverse events during the first trimester, and vaccination pre-conception may have an effect on the developing foetus.[77] Other studies also had participants who were vaccinated prior to conception, but these results could be separated from results specific to women vaccinated during pregnancy.[78, 79] The initial search was conducted during November 2012, and during the course of the review up until 29th of April 2013 six studies published after the initial search were identified through Embase and Pubmed email alerts using the original search criteria.[18, 79-83] These were critically assessed and included in the review. The 35 full text articles excluded are described in Appendix IV. Figure 1 outlines the process used to identify studies for inclusion in this review.

A total of 39 studies were retained in the review,[7, 18, 37, 46, 53-59, 77-104] with six studies including outcomes on the effectiveness of maternal influenza vaccination for pregnant women,[7, 18, 59, 80, 89, 95] and seven for their infants up to six months of age.[37, 58, 59, 84, 93, 95, 99] Foetal and infant outcomes, as either primary or secondary measures were present in 28 studies.[7, 18, 46, 53, 54, 57, 77-81, 83, 85, 87-89, 91-98, 100-103] Adverse event outcomes for pregnant women were present in 24 studies.[53-59, 77, 78, 81-83, 86-90, 92-95, 97, 103, 104] There were 14 studies that were investigating outcomes for trivalent seasonal influenza vaccination.[7, 37, 55, 59, 79, 82, 84, 89, 95, 96, 99, 101, 102, 104] Two of these were reporting on outcomes of the same study population;[59, 102] and different outcomes were extracted and not duplicated in the synthesis. The majority of studies included in this review were investigating the effects of the monovalent influenza A (H1N1) 2009 vaccine, with 22 in total.[18, 46, 53, 54, 56, 58, 77, 80, 83, 85-88, 90-94, 97, 98, 100, 103] One study contained outcomes for both influenza A (H1N1) 2009 and the trivalent seasonal influenza vaccine.[81] Monovalent (Hsw1N1) was the only other

single antigen vaccine and two studies contained outcomes pertaining to this vaccine.[57, 78] The types of vaccines and outcomes are described in table 3.

The publication dates of the included studies ranged from 1964 to 2013, with 46% of the studies being published during or since 2012. Some of the studies that investigated the effectiveness and safety of the trivalent seasonal vaccine spanned three or more influenza seasons.[7, 37, 79, 84, 95, 99, 101] Randomised controlled trials only made up three of the studies included in this review.[55, 56, 59] Of those, only one assessed effectiveness of the influenza vaccine at reducing influenza and influenza-like illness.[59] The remaining two studies were not studies of effectiveness or compared to a non-vaccinated cohort and were included for adverse event outcomes only.[55, 56] Each study was randomised to a different control intervention which included pneumococcal vaccine,[59] tetanus toxoid,[55] and two doses of the influenza vaccine.[56] Steinhoff et al. (2012),[102] conducted secondary analysis on the same data set used by Zaman et al. (2008).[59] This review only extracted previously unpublished adverse event data.

Observational cohort studies made up the majority of studies in this review. Of these, seven were prospective cohort designs,[37, 54, 57, 77, 78, 88, 94] two were prospective studies with a sub-population of pregnant women who were compared to other populations in the study,[86, 90] and six were single arm studies that presented data as frequencies and percentages.[53, 58, 92, 97, 103, 104] The remainder of studies consisted of 14 retrospective cohort studies,[7, 18, 46, 80-82, 85, 91, 93, 95, 96, 98, 100, 101] three of which were case-control studies,[79, 84, 99] one was a cross sectional design,[83] and two were mixed methods.[87, 89]

The study cohorts were mainly from the USA and Europe, with 17 studies undertaken in the USA,[7, 37, 55-57, 78-82, 84, 89, 95, 96, 99, 101, 104] one each from Canada[85] and Argentina,[83] and 14 from Europe[18, 46, 53, 54, 58, 77, 86, 87, 91, 97, 98] and the UK.[94, 100, 103] Five studies were conducted in the Asian region.[59, 88, 90, 92, 93] The randomised controlled trial conducted in Bangladesh was only counted once.

The characteristics of the studies included in this review are described in more detail in Appendix V.

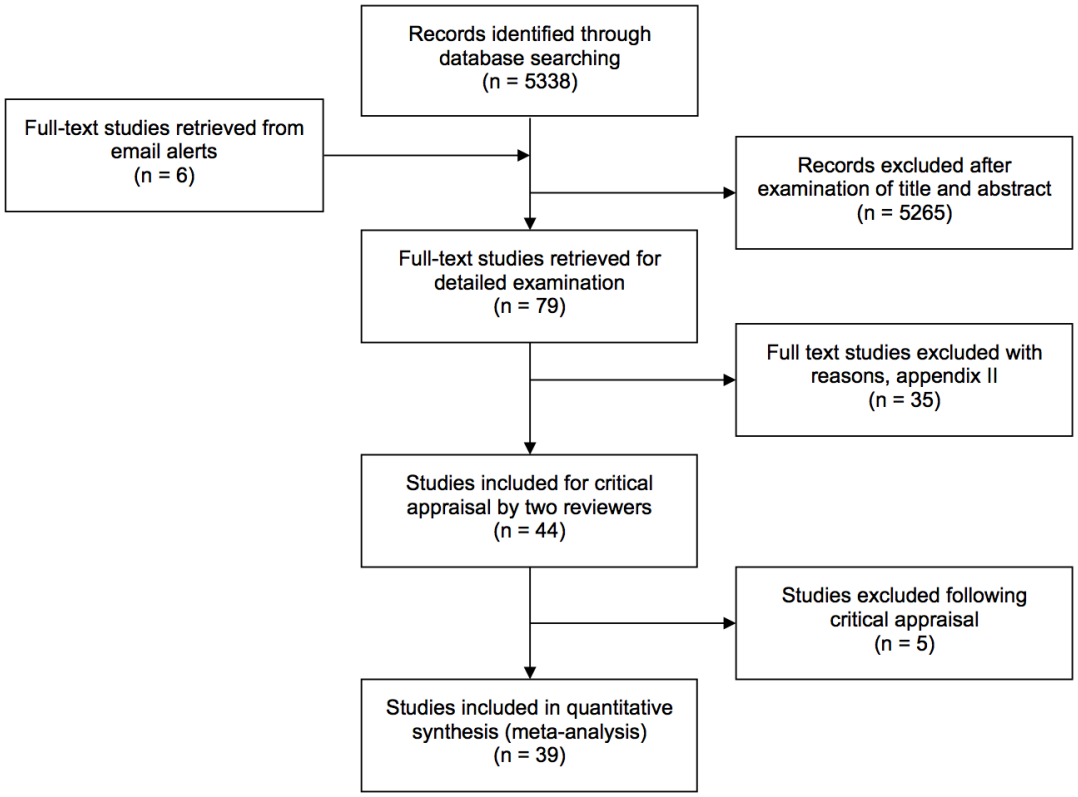

Figure 1 Identification and selection of studies

Table 3 Number of included studies per outcome and vaccine type

Vaccine	Effectiveness on mother	Effectiveness on infant <6 months	Safety for pregnant women	Safety for foetus
Trivalent seasonal	4	5	6	7
Monovalent A (Hsw1N1) 1976	0	0	2	2
Monovalent A (H1N1) 2009	2	2	16	19

3.1.1 Assessment of Methodological quality

The included studies vary from moderate to high quality studies, ranging from 5 criteria to 9 criteria on critical appraisal. Randomised /Pseudo-randomised control trials were generally performed well, with description of blinding to treatment allocation being the lowest rated criteria. The only randomised controlled trial investigating the effectiveness of the vaccine was of high quality meeting nine of the 10 criteria.[59] As previously described, retrospective and prospective cohort studies, case control and cross sectional studies made up the majority included in this review. Most categories were well performed, although describing the outcomes of the people who withdrew from the prospective cohort studies was missing in all studies that had attrition of participants.[77, 78, 87, 89, 94] Identifying confounding factors and

including strategies to deal with them was performed in most studies, however the number and type of variables used in analysis varied widely between studies and need to be considered on an individual basis when assessing outcomes. These are described in more detail in the description of relevant outcomes. Descriptive studies did not contain random samples of pregnant women and dealt poorly with potential confounding factors, although two studies only included healthy pregnant women.[58, 92] Description of outcomes for people who withdrew from the studies was also not well performed.

Some of the larger retrospective cohorts contained close to entire populations of pregnant women in a country or region and thus enabled investigation of potentially rare outcomes in the participants.[18, 46, 85, 91, 98] The majority of these studies collected information from medical databases and relied on the accurate coding of ICD codes, or documentation of birthing outcomes using other regional or national databases. This method of data collection can be susceptible to missing and misclassification of data. Two retrospective cohorts collected data directly from client notes and discharge summaries,[93, 101] and one from information collected on a database following direct patient contact and questioning.[96]

Observational studies included in this review had multiple 'all cause' outcomes. All cause outcomes of premature birth, small for gestational age, congenital abnormalities, foetal death, spontaneous abortion, and low birth weight are contained in this review, without indication that the pregnant women had influenza at any stage during their pregnancy. Even with regression analysis, propensity matching and adjustment for potential confounding factors, there are limits as to how this data can be interpreted.[105] There are numerous confounders that may increase the risk of some of these outcomes including maternal age, smoking, prior pre-term birth, short cervical length, low socio-economic status, African ancestry, alcohol or drug use, and chronic disease such as hypertension, renal insufficiency or diabetes.[106, 107] The risk of residual confounding is inherent in the nature of retrospective observational cohort studies.[108] This has been shown to be the case with previous influenza research in which observational research has been used [105, 109], and needs to be taken into consideration when interpreting the results, especially considering the number of observational studies in this review.

Influenza vaccine comes in many different compositions, due to the regular changes in the antigenic make-up the vaccine. Even with the subgroup of influenza A (H1N1) 2009 vaccines, there are different adjuvants used, as well as differences in the method of manufacture, with split-virion and subunit vaccines produced. The match of the vaccine to the circulating strain of influenza virus during the study period is an important factor when assessing the effectiveness of influenza vaccines and comparing studies. This was scarcely reported in the papers included in this review, although surveillance suggests that there was a good match between the influenza A (H1N1) 2009 vaccine and the studies conducted during the pandemic period.[110-112]

On the whole, the sampling of participants in the cohort studies was representative of the pregnant

population during their second or third trimester of pregnancy due to large population-based cohorts. Women were included with other factors that placed them or their foetus at risk from complications from influenza. Several studies were performed on healthy pregnant women only.[55, 56, 58, 59, 87, 88, 92, 113] Pregnant women vaccinated during their first trimester were under represented in the study cohorts and this has been considered in the analysis.

There are 16 studies included in this review in which one or more of the authors declared receiving pharmaceutical industry support or payment. Potential conflicts of interest were unclear in seven studies, with most being from the older published material, and the remaining 16 reported no conflict of interest. Overall eight studies did not report on whether they obtained ethical or research review board approval,[7, 55, 57, 78, 84, 89, 90, 97], three reported that they were using surveillance data that did not require ethics approval in their country,[46, 91, 98] one reported that they did not require ethics without any rationale,[86] and the remainder reported seeking approval.[18, 37, 53, 54, 56, 58, 59, 77, 79-83, 85, 87, 88, 92-96, 99-104]

Reviewer agreement was high and discrepancies were resolved following discussion. Further detail regarding methodological quality is tabled in Appendix VI and discussed in section 4.7.

3.1.2 Assessment of heterogeneity

The clinical and methodological diversity reduced the number of studies able to be combined in meta-analysis with the majority of studies presented in narrative form.

No studies assessing the effectiveness of the vaccine, or adverse events in pregnant women were suitable for meta-analysis due to heterogeneity of the studies or definitions of the outcomes. There were four birthing outcomes that were consistently defined and suitable to be combined in meta-analysis, namely premature birth less than 37 weeks, very premature birth less than 32 weeks, SGA less than 10th percentile infants, and low birth weight less than 2500g.

There were three studies that were assessed as being homogenous in their methodological design and appropriate to be combined in meta-analysis. Each study contained close to complete populations of pregnant women with singleton births in a country or large city, reducing potential sampling bias. All ran for one influenza season and were investigating outcomes for the influenza A (H1N1) vaccine.[46, 85, 91] They all adjusted for some potential confounding variables including maternal age and maternal smoking, as well as having similar methods in collecting data. The only other large country-wide population based cohort study used a Cox proportional hazard model with an underlying time metric, and was unable to be combined with the previous mentioned studies for this reason.[18] The remaining retrospective cohort studies that assesses these outcomes were excluded from meta-analysis because they were not large population based cohort studies. There were no prospective designed studies that were homogenous and suitable to combine.

3.2 Effectiveness in pregnant women

3.2.1 Pregnant women with a self-reported respiratory illness

Only two studies examined the effectiveness of reducing self-reported influenza-like illness and both were for the seasonal vaccine. The double blind randomised controlled trial by Zaman et al. (2008) investigated a split-virion seasonal vaccine and met nine of the 10 critical appraisal criteria. The study participants lived in Bangladesh and were healthy pregnant women in their third trimester of pregnancy. In the study 172 were vaccinated with the influenza vaccine and 168 of the control group received the pneumococcal vaccine. The mean age was 25.1 years with a range of (18.0 to 36.0) in the vaccinated group and 24.9 years with a range of (18.0 to 36.0) in the control group. The primary outcome for the study was laboratory confirmed influenza in infants. Incidence rate ratios (IRR) were calculated for the study outcomes with the use of Poisson regression models and estimates of clinical effectiveness were calculated with the formula (1 - IRR) x 100. They measured self-reported respiratory illness with any fever, with a clinical effectiveness of 35.8% (95% CI: 3.7 to 57.2) and a risk difference of -14.2 (95% CI: -25.5 to -2.9, $p<0.05$).[59] The study also included respiratory illness with fever over 38°C, with a clinical effectiveness of 43.1% (95% CI: -9.0 to 70.3) and a risk difference -7.3 (95% CI: -14.5 to - 0.1, $p<0.05$).[59] Numbers needed to vaccinate were calculated using the risk difference reported in the study and it is estimated that 7 (95% CI: 4.0 to 34.5) women need to be vaccinated to prevent one case of self-reported influenza with any fever and 14 (95% CI: 6.9 to 1000) women to prevent one case with a fever over 38°C. The antigenic match of the vaccine to the circulating strains during the study period was not reported in the paper. However, a secondary analysis also included in this review reported a substantial number of influenza A (H3N2) and B viruses were in circulation during a part of the study period.[102] It is not possible to accurately ascertain how well matched the vaccine was although it did contain an influenza A (H3N2) strain and influenza B virus strain. It is also unclear how the study period was chosen in relation to influenza circulation in the community. The authors report that the influenza virus was circulating for 10 of the 11 months of observation.[59]

Hulka (1964) conducted a mixed prospective and retrospective cohort study.[89] Pregnant women were administered a seasonal whole-virion influenza vaccine or saline placebo. It is unclear how these groups were allocated and the study was timed to run during the putative influenza season. Some women were added retrospectively to the control group if they were not vaccinated during the previous winter and then asked if they experienced “the flu with fever”.[89] Data were presented as frequencies and percentages. The author originally planned to conduct a double blind trial, but technical difficulties and the imminent arrival of the influenza season meant that the investigators were not blinded to allocation participants.[89] Study participants were mostly from a low socioeconomic background and consisted largely of an African American population. No other characteristics of the cohorts were described.

Pregnant women in any trimester of pregnancy were included and 19 women were vaccinated in their first trimester. There were 316 women vaccinated with the influenza vaccine and 138 with the placebo. There was a marked attrition rate with the drop out of 149 women from the vaccinated cohort and an unknown number from the control group. Self-reported influenza-like illness and cases were identified via mail, phone, or personal visits during winter.[89] In the influenza vaccinated group 24 (11%) pregnant women reported a fever and upper respiratory disease compared to 36 (20%) pregnant women in the unvaccinated control group and this was reported as not being significantly different.[89] The whole-virion vaccines are considered to be more immunogenic than the split-virion and subunit vaccines currently available,[31] so the outcome may not be generalisable to current subunit or split-virion vaccines. The attrition of a large number of participants and retrospective allocation participants to the control arm of the study limits the findings that can be made from this study.

3.2.2 Pregnant women with an Influenza-like illness at outpatient or clinic visit following exposure to influenza vaccine

Seasonal trivalent influenza vaccine was investigated in three studies.[7, 59, 95] Zaman et al. (2008) was the only randomised controlled trial on the effectiveness of reducing clinic visits.[59] They defined a case as a clinic visit with respiratory illness and fever and reported clinical effectiveness.[59]

Effectiveness of seasonal influenza vaccines was investigated by two retrospective cohort studies using ICD-9 codes to identify influenza cases.[7, 95] Coding was used to identify a variety of respiratory illnesses and this reduces the specificity of their results. Black et al. (2004) followed up all women with live births during Influenza seasons from 1997 to 2002 in northern California.[114] The period was identified with the first and the last virus isolation in the community. The study was unclear about the match of the vaccine with circulating strains during these periods. Over this period they followed up 3707 women vaccinated during pregnancy and compared outcomes with 45878 unvaccinated women who were pregnant during the same period. The characteristics of the women were not described and the trimester of vaccination was not reported. The influenza vaccination status of women in the cohort was determined through review of an Immunisation Tracking System database. The cohorts were unmatched and limited potential confounders were considered. Effectiveness for pregnant women having an influenza-like illness at an outpatient visit was assessed using hazard regression analysis and adjusted for women's age and week of delivery.[114]

Munoz et al. (2005) conducted a retrospective cohort study over five influenza seasons from July 1, 1998, to June 30, 2003 in Houston Texas.[95] Prevalent strains are listed in the paper, however the matches of the vaccines to the circulating influenza strains are not stated. The vaccinated cohort was made up of 225 healthy pregnant women vaccinated in their second or third trimester. They were included in the study if they had an uncomplicated singleton pregnancy and at least one prenatal care

visit. The mean age of the vaccinated cohort was 30.7 years and the mean gestational age at vaccination was 26.1 weeks.[7, 95] The comparison cohort of 826 unvaccinated pregnant women was matched for maternal age at delivery, month of delivery, type of insurance, and had a mean age of 30.8 years.[95] There was no adjustment of any potential confounding variables. Nominal values were compared among the groups with chi-squared test or Fisher exact test. Retrospective studies are not as reliable as prospective studies for assessing effectiveness of vaccines.[115] The non-specific nature of the case identification with ICD-9 codes impacts on the accuracy of identifying influenza cases for both retrospective studies.

Influenza-like illness outcomes for the influenza A (H1N1) 09 vaccine were contained in two retrospective cohort studies.[18, 80] Hårberg et al. (2013) conducted a study primarily investigating any potential association between the influenza vaccine and foetal death, with a secondary outcome measure of 'primary care physician diagnosed influenza cases'.[18] The study included nearly all women from Norway who were pregnant and had a singleton birth in 2009 and 2010, with 25976 pregnant women vaccinated during any trimester, and 87335 in the unvaccinated cohort.[18] The study cohort consisted of 80% of women aged less than 35 years and 11% of women vaccinated had a chronic illness. There were 57% in the vaccinated cohort compared to 43% in the unvaccinated cohort with a chronic illness. The study used a Cox proportional hazard model using a time dependent variable from the day of gestation to the day of clinical diagnosis, or day of delivery, or end of study period. The period selected to measure the effectiveness of the vaccine was based on laboratory-confirmed cases and physician visits for influenza that were reported to the Norwegian Institute of Public Health.[18] Only pregnancy days that fell during a 3-month period of the main pandemic wave were included in this outcome.[18] Selecting a period when the peak circulation of the virus is occurring means that the vaccine has its best chance of showing a reduction in influenza cases, rather than a putative period that may contain periods of low or no virus circulation.

Richards et al. (2013) were primarily investigating the impact of the influenza vaccine on birthing outcomes during the 2009 influenza A (H1N1) pandemic in a health consortium located in California USA.[80] Their study included third trimester births only with first and second trimester births being excluded. The mean age of participants was 31.2 years, with a standard deviation (SD) ($\pm$5.6). The study ran over a 12-month period and included 1125 vaccinated women and 1581 unvaccinated women; the authors were contacted and data were not collected on which trimester they were vaccinated. The study period was derived from the first positive influenza A (H1N1) 09 case in the region up to when the cases dropped below 5%.[80] It is not clear if the unvaccinated cohort had a larger proportion of participants who were exposed to the peak periods of virus circulation, as some participants were assigned a cohort prior to the introduction of the vaccine and thus could only be in the

unvaccinated cohort. The analysis did not involve assessing the time spent at risk using a time dependent hazard ratio, or match or adjust for confounding variables. The vaccine effectiveness was calculated as one minus the odds ratio (OR) X 100. If the unvaccinated cohort did have a higher amount of exposure to the peak influenza period, this would result in a bias towards the vaccinated cohort.

The sole randomised controlled trial indicated that the influenza vaccination during pregnancy resulted in a statistically and clinically significant reduction in respiratory illness with fever.[59] However, the sample size and absence of other randomised controlled trials means that the estimate of the numbers needed to treat is not precise. Both retrospective studies on the trivalent vaccine used methodology not conducive to accurately estimating effectiveness of the vaccine.[7, 95] There was no statistically or clinically significant evidence that trivalent influenza vaccine reduced clinic visits for influenza-like illness. The two retrospective studies on the influenza A (H1N1) 2009 vaccine estimated a statistically significant reduction.[18, 80] Ideally prospective studies would be used to establish effectiveness of an intervention such as a vaccine.[105] Under the circumstances of a sudden pandemic outbreak that restricts the opportunities to conduct such a study, the use of a hazard model and a concise influenza period has resulted in a high quality retrospective study that may be clinically important for assessing the appropriate management of pandemic influenza in pregnant women in the future.[18] The results are described in further in table 4.

Table 4 Pregnant women with an Influenza-like illness at outpatient or clinic visit following exposure to influenza vaccine

Study	Design	Vaccination group (n)	Control group (n)	Results	Definition of influenza or influenza-like illness
Black et al. (2004)[7]	Retrospective cohort	Trivalent (3707)	No vaccine (45878)	Hazard ratio (HR) 1.151 (p=0.088), adjusted for women's age and week of delivery.	Diagnostic coding identifying one or more of the following; upper respiratory infection, pharyngitis, otitis media, asthma, bronchial asthma, viral infection, pneumonia, fever, cough, and wheezing associated with respiratory illness.
Munoz et al. (2005)[95]	Retrospective cohort	Trivalent (225)	No vaccine (826)	51 (22.6%) with acute respiratory infections in vaccinated group and 156 (18.9%) in control group (p=0.24).	ICD-9 codes for unspecified viral infection, acute respiratory infections, other diseases of the respiratory tract, pneumonia, and influenza.
Zaman et al. (2008)[59]	Randomised controlled trial	Trivalent (172)	Pneumococcal vaccine (168)	Clinical effectiveness 24.9% (95% CI: −43.9 to 60.8)	Clinic visit with respiratory illness and fever
Hårberg et al. (2013)[18]	Retrospective cohort	Monovalent H1N1 (25976)	No vaccine (87335)	Adjusted HR 0.30 (95% CI: 0.25 to 0.34). It is unclear what variables were included.	R80 code from a primary care physician, which can be a set of influenza-like illness symptoms or confirmed by culture or serology.
Richards et al. (2013)[80]	Retrospective cohort	Monovalent H1N1 +/- trivalent (1125)	No vaccine (1581)	Effectiveness against diagnosed influenza A (H1N1) 2009 infection. 61.5% (95% CI: 15.5% to 82.5%).	Reverse transcription polymerase chain reaction test positive for influenza, or having a medical visit during pregnancy with influenza-related ICD-9 diagnosis.

3.3 Effectiveness in infants up to 6 months of age

3.3.1 Influenza or influenza-like illness identified in non-hospitalised infants up to 6 months of age.

Effectiveness of the vaccine in preventing influenza-like illness in non-hospitalised infants was contained in three studies investigating the trivalent seasonal vaccine,[37, 59, 95] and one with outcomes for the influenza A (H1N1) 09 vaccine.[58]

Zaman et al.'s (2008) randomised controlled trial was described earlier and the effectiveness of the vaccine in infants up to 6 months of age was the study's primary outcome measure.[59] The same method of calculating the effectiveness for pregnant women was used for infants less than 6 months. Following vaccination, mothers were asked to record axillary temperatures of their infants. Families were also asked to bring infants who were ill to the study clinic for assessment, influenza-antigen testing and treatment.[59]

Eick et al. (2011) conducted a prospective cohort study on an Apache Indian reservation in Arizona USA. Navajo and White Mountain Apache Indian mother-infant pairs were recruited after delivery over 3 influenza seasons from November 2002 to September 2005.[37] It is not clear how these periods were decided. Infants included in the study consisted of 51% male and 49% female and no other infant characteristics were described. Pregnant women were vaccinated during their second or third trimester and 573 were enrolled in the vaccinated cohort and 587 in the unvaccinated cohort.[37] Active surveillance was then conducted and included review at the clinic, emergency department, and inpatient paediatric wards until the child reached 6 months of age. Participants of both groups were similar for sex, presence of household smokers, day-care, gestational age, and mean birth weight. There was a statistically significant difference in infants who were breastfed with more in the vaccinated cohort and more residing in a house with a coal-burning stove.[37] These variables were assessed and no significant associations were found and there were no adjustments made to the final results.

The retrospective study of Munoz et al. (2005) has also been described previously. They used ICD-9 respiratory illness codes reported during ambulatory medical visits in the first 6 months of life as described in table 2.[95]

The only study on the influenza A (H1N1) 09 vaccine was a phase two single arm clinical trial primarily investigating immunologic effects and safety of the vaccine, with data being presented as frequencies and percentages.[58] Participants were healthy pregnant women between 22 and 32 weeks of gestation, with a median age of 32.0 years (interquartile range 30.1 to 36.4). Pregnant women were enrolled in the study and they filled out questionnaires at 1 and 6 months after birth about their infant, including information about influenza-like symptoms. No laboratory confirmation of influenza-like illness was

performed.[58]

The number needed to vaccinate for Zaman et al (2008) was calculated at 16 (95% CI: 8.2 to 200) to prevent one case of laboratory confirmed influenza in an infant up to 6 months of age. Eick et al. (2011) estimated that the reduction in risk of an infant presenting with laboratory confirmed influenza was 41% (95% CI: 7% to 63%).[37] Munoz et al. (2005) relied on non-specific coding and this is a low quality method of measuring effectiveness.[95] Both prospective studies by Eick et al. (2011) and Zaman et al. (2008) indicate that infants of vaccinated mothers up to 6 months of age have a clinically and statistically significant reduction in risk of being diagnosed with laboratory confirmed influenza. Both of the studies from Zaman et al and Eick et al. were conducted on groups of people who were either in a developing country, or considered to be a vulnerable population group, possibly affecting the generalisability of the results. Clinical and methodological diversity of the studies did not allow meta-analysis to be performed. The studies and results are described further in table 5 and Appendix V.

3.3.2 Influenza or Influenza-like illness in infants up to 6 months of age that required hospital admission.

Hospitalisation of infants up to 6 months of age was investigated in three studies of the trivalent seasonal influenza vaccine.[37, 84, 99] Benowitz et al. (2010) conducted a case control study that included pregnant women vaccinated in their second or third trimester over a period of nine influenza seasons up to, but not including, the 2009 pandemic. Case and matched controls were enrolled in a large metropolitan hospital in the USA.[84] Risk set sampling was used to match hospitalised infants with a positive influenza direct fluorescent antibody test with controls that were admitted with influenza-like illness, but tested negative for influenza. In the case cohort 11.6% were born premature compared to 19.3% in the control group. Both groups had similar number of infants with a chronic medical condition with 36.3% in the case group and 38.5% in the control group. The most notable differences were that case subjects came from households with a larger number of people residing in them (p=0.015), and they were also significantly less likely to live with anyone who had been vaccinated for influenza (p=0.001).[84] Matched odds ratios were estimated for vaccination of mothers of the 91 case subjects, compared with mothers of the 156 matched control subjects. Effectiveness of the vaccine was calculated as 1 minus the matched odds ratio times 100. Logistic regression was performed for multiple potential confounders and the adjusted model retained vaccination of household contacts and prematurity.

Poehling et al. (2011) conducted a case control study that ran over seven influenza seasons up to, but not including, the 2009 influenza pandemic season. The study took place in hospitals situated in 3 different US counties and the trimester that women were vaccinated was not stated.[99] Infants were identified through hospital surveillance and were eligible for enrolment if they were hospitalised with

fever and/or acute respiratory symptoms during the winter. All children enrolled had nasal and throat swabs for viral culture or Polymerase chain reaction (PCR) testing for influenza A and B. The infants who had an influenza positive test comprised the case group and the control group consisted of those with a negative influenza test. The vaccinated cohort consisted of mothers from Hispanic (22%), African American (15%), and Caucasian (21%) backgrounds. Infants with private insurance were more likely to be from vaccinated mothers. Overall infants included in the study consisted of 44.0% female, 56% male, 11.8% who were born premature, and 7.9% who had a high-risk condition.[99] The trimester that the mother was vaccinated in during pregnancy was not reported. Multivariate logistic regression models were used and consisted of three different models.

Eick et al.'s (2011) prospective study has been previously described.[37] All three studies met seven or more of the appraisal criteria. The methodological diversity and clinical heterogeneity prevented meta-analysis being performed.

A retrospective cohort study investigating the influenza A (H1N1) 2009 vaccine by Lin et al. (2012) was conducted in Taiwan and consisted of 202 women vaccinated during pregnancy, with 10 (4.9%) in the first trimester. The infants were only followed up for 8 weeks post-partum and consisted of 14 cases in total.[93] The mean age for both cohorts was 32.4 years (SD±4.0) and 32.8 years (SD±3.9), and the mean gestational age at vaccination was 26.5 weeks (SD±8.0). Data were presented as frequencies and percentages for this outcome and the study was well conducted and met all the criteria for critical appraisal.

Both case-control studies on the trivalent seasonal influenza vaccine indicated a clinically and statistically significant reduction in influenza related hospitalisation of infants less than 6 months of age following vaccination of their mothers during pregnancy.[84, 99] The prospective study by Eick et al. (2011) indicated that vaccination during pregnancy resulted in statistically and clinically significant protection from influenza complications.[37] Results from each study and definitions are described in table 6.

Table 5 Influenza or Influenza-like illness identified in non-hospitalised infants up to 6 months of age.

Study	Design	Vaccination group (n)	Control group (n)	Results	Definition of Influenza or Influenza-like illness
Eick et al. (2011)[37]	Prospective cohort	Trivalent (573)	No vaccine (587)	Laboratory confirmed influenza virus infection. Relative Risk (RR) 0.59 (95% CI: 0.37 to 0.93) Influenza-like illness RR 0.92 (95% CI: 0.73 to 1.16)	Laboratory confirmation was achieved by virus culture, serology, or rapid influenza diagnostic test. Influenza-like illness was defined as a medical visit with at least one of the following: fever ≥ 38.0°C, diarrhoea, or respiratory symptoms.
Munoz et al. (2005)[95]	Retrospective cohort	Trivalent (225)	No vaccine (826)	26.8% of vaccinated group 30.2% of unvaccinated group	ICD-9 diagnostic codes for acute respiratory tract illness.
Zaman et al. (2008)[59]	Randomised controlled trial	Trivalent (169)	Pneumococcal vaccine (167)	Respiratory illness with any fever, 28.9% (95% CI: 6.9 to 45.7) Respiratory illness with temperature >38°C, 28.1% (95% CI: - 4.6 to 50.6) Clinic visit, 42.0% (95% CI: 18.2 to 58.8) Influenza test positive, 62.8% (95% CI: 5.0 to 85.4)	As described in results column. Influenza test positive was conducted using rapid test for both influenza A and B.
Tsatsaris et al. (2011)[58]	Single arm prospective	Monovalent H1N1 09 (116)	N/A	28 infants had fever associated with another respiratory symptom during the study period.	Self-reported fever associated with another respiratory symptom during the study period via a questionnaire.

Table 6 Influenza or Influenza-like illness in infants up to 6 months of age that required hospital admission.

Study	Design	Study group (n)	Control group (n)	Results	Definition Influenza or Influenza-like illness
Benowitz et al. (2010)[84]	Case-control	Influenza test positive (91)	Influenza test negative (156)	Adjusted effectiveness of the vaccine, 91.5% (95% CI: 61.7% to 98.1%, p=0.001) Adjusted model retained, immunisation of household contacts and prematurity	Positive for influenza by direct fluorescent antibody test.
Eick et al. (2011)[37]	Prospective cohort	Trivalent (573)	No vaccine (587)	Unadjusted RR 0.61 (95% CI: 0.45 to 0.84)	Influenza-like illness requiring hospitalisation, with at least one of the following signs or symptoms reported: fever $\geq$ 38.0°C, diarrhoea, or respiratory symptoms.
Poehling et al. (2011)[99]	Case-control	Influenza test positive (151)	Influenza test negative (1359)	Adjusted OR 0.52 (95% CI: 0.30 to 0.91) Adjusted model included age, sex, race/ethnicity, site, study year, tertile of the influenza season, smoke exposure at home, number of siblings, day care attendance, insurance status, and whether the infant was ever breast-fed.	Positive viral culture or PCR test.
Lin et al. (2012)[93]	Retrospective cohort	Monovalent H1N1 09 (202)	No vaccine (206)	6 (3.0%) vaccinated 8 (3.9%) unvaccinated	Upper respiratory tract infection documented in medical records.†

† Infant health status data were only followed until 8 weeks post-partum.

3.4 Vaccine safety

3.4.1 Adverse events for pregnant women

Studies reporting on adverse events included 18 investigating monovalent influenza A (H1N1) vaccines[53, 54, 56-58, 77, 78, 81, 83, 86-88, 90, 92-94, 97, 103] with six including data on the seasonal trivalent vaccine.[55, 59, 82, 89, 95, 104] An assortment of methods and measures were included in the identification of adverse events for pregnant women. Prospective methods were used in 19 of the 24 studies and they included active surveillance via direct interviews, questionnaires, phone contact, email contact, and also identified serious adverse events through surveillance of medically attended events.[53-59, 77, 78, 86, 88, 90, 92-94, 97, 103, 104] The majority of studies presented a descriptive summary of adverse events with data presented as frequencies and percentages, or as a narrative summary for one or both of their cohorts.[53-59, 77, 78, 88, 92-94, 97, 103, 104] Three prospective studies contained pregnant women as a sub-population of other influenza vaccinated groups,[86, 90, 94] and two of these compared the risk of an adverse event with other 'at risk' populations using multivariate logistic regression models.[86, 90] The quality of the prospective studies is mixed, with attrition of participants in some studies a concern.[77, 78, 86, 87, 89, 92, 97]

Other studies used mixed designs,[87, 89] with one comparing adverse events from a whole-virion vaccine with a placebo,[89] and the other estimated an adjusted odds ratio for preeclampsia.[87] Only four studies with outcomes for adverse events were retrospective,[81-83, 93] with three describing frequencies and percentages of adverse events from medical presentations.[81, 83, 95] One study performed a retrospective analysis of medically attended adverse events over a seven year period and calculated rates compared to non-vaccinated pregnant women.[82] In all there were nine studies that only contained vaccination to pregnant women in their second or third trimester.[53-59, 86, 95]

Characteristics of participants involved in the studies varied, with seven made up of healthy pregnant women, [55, 56, 58, 59, 87, 88, 92, 95] four others contained between 3.7% to 17.6% of pregnant women with a comorbidity,[78, 82, 95, 103] and others did not describe an overall percentage of women with a comorbidity.[53, 54, 57, 77, 81, 83, 86, 89, 90, 93, 94, 97, 104] The make-up of the vaccine also varied between studies with the adjuvant and formulation not always described.[55, 59, 81, 82, 94, 95] Studies that did describe the type of vaccine included subunit MF-59-adjuvant,[53, 54, 83, 86, 87] split-virion adjuvant free,[56, 58, 77, 88, 90, 92, 93, 97, 104] split-virion AS03-adjuvant,[77, 103] and whole virus vaccines.[57, 78, 89]

The methods and characteristics of each study are described in more detail in Appendix V and methodological quality has been described previously and can also be found for individual studies in Appendix VI.

The largest study on the trivalent seasonal vaccine was conducted by Nordin et al. (2013) and used ICD-9 codes from inpatient, outpatient, or emergency department visits to assess medically attended

visits within 3 days of being vaccinated.[82] The study population consisted of 10 health care systems across the USA and contained 21553 women vaccinated during their first trimester. The mean age was 30.8 years (SD±5.6) and the vaccinated cohort were more likely to have a pre-existing condition (14.5% compared with 11.7%).[82] Unvaccinated and vaccinated women were matched using an algorithm to the age at pregnancy (±1year) and pregnancy start date (±30 days). Presentations that were included were based on biological plausibility of the timing of the vaccine being associated with an adverse event and this included two different time periods; 3 days post vaccination and 1- 42 days post vaccination. The authors reported that rates for medically attended events 0–3 days after vaccination were low and did not exceed 2 per 10,000 among both the vaccinated and unvaccinated women[82]

There was minimal data available for adverse events for pregnant women who received the trivalent seasonal vaccine, including local reactions such as pain and systemic reactions such as fever, myalgia, and fatigue. For both the seasonal and influenza A (H1N1) 2009 influenza vaccine the most common adverse events were local symptoms, which included pain and/or redness at the injection site. Systemic symptoms such as fever, headache, myalgia, malaise, and nausea were also reported in smaller numbers. For the influenza A (H1N1) 09 vaccine the studies that individually itemised adverse events had rates that ranged from 19% to 41.6% for local pain, 1% to 13.7% for fever, 9% to 30% for headache, 22% to 40% for fatigue, 2.7% to 23.7% for myalgia, and 2% to 14.2% for cough and respiratory symptoms.[53, 56, 92, 97, 103]

There were two studies that used multivariate logistic regression models to compare the risk of an adverse event in pregnant women following vaccination with monovalent influenza A (H1N1) 09 vaccine with other vaccinated groups.[86, 90] The study by Hårmark et al.[86] was conducted in General Practitioner settings in the Netherlands and was attempting to quantify adverse events related to an influenza A (H1N1) subunit MF-59-adjuvant vaccine. The prospective study investigated adverse events via questionnaires of all groups in receipt of the vaccine, including 72 pregnant women.[86] The characteristics of the pregnant women are not described. They performed multivariate logistic regression as described in table 7. Hwang et al. (2011) used a similar methodology to assess the risk to pregnant women. With only 21 pregnant women in their study the lack of statistical precision due to the small sample sizes in both studies limits the conclusions that can be made.[90]

Both showed an increased risk for pregnant women experiencing adverse events compared to other vaccinated groups, including age groups, and groups with a comorbidity, with Hårmark et al.[86] estimating a statistically significant increase. However, the small number of pregnant women and attrition of participants means that these results should be interpreted with caution. The results are contained in table 7.

There were four studies that reported on rates of preeclampsia following vaccination with influenza A

(H1N1) 09 vaccine.[77, 81, 83, 87] Three of those compared rates with an unvaccinated cohort.[77, 83, 87] Two of those calculated an odds ratio and had a point estimate that favoured the unvaccinated cohort. [77, 87] None indicated a statistically or clinically significant association with vaccination.

There were no adverse events of special interest such as Guillain-Barré Syndrome, other rare neurological conditions or deaths reported from the 24 studies included. Serious adverse events such as hospitalisation were mostly associated with adverse pregnancy outcomes and not necessarily associated with vaccination. The studies that further investigated adverse pregnancy outcomes found no relationship with the vaccine.[58, 97]

Overall the results are clinically significant to the extent that pregnant women and health care providers need to be aware of the incidence of minor local and systemic reactions in order to obtain appropriate consent and provide advice on the management of adverse reactions post vaccination. There is no clinically or statistically significant evidence that influenza vaccination has been responsible for serious adverse events in these study populations. The size, heterogeneity, and design of the studies means that it is not possible to draw any findings about the association a particular trimester of pregnancy, or type of adjuvant, or formulation may have on adverse event outcomes for pregnant women.

Meta-analysis was not suitable for adverse event outcomes due to the clinical and methodological diversity of the studies. Further description of the included studies can be found in table 7 and 8, as well as the studies designs and methods in Appendix V.

Table 7 Adverse events in pregnant women following influenza vaccination.

Study	Design	Vaccination group (n)	Control group (n)	Adjuvant & formulation	Results
Englund et al. (1993)[55]	Pseudo-randomised controlled trial	Trivalent (13)	Tetanus toxoid (13)	Not reported	No significant adverse reaction including fever, moderate or severe pain, or need to visit a physician were reported.
Hulka (1964)[89]	Mixed prospective retrospective cohort	Polyvalent (363)	Placebo (181)	Whole-virion, adjuvant not reported	40% of patients receiving saline complained of pain and 83% of vaccinated reported pain. 43% is therefore potentially attributable to the vaccine. Malaise was experienced by 15-20% of vaccinated women and only 1.9% of unvaccinated women.
Munoz et al. (2005)[95]	Retrospective cohort	Trivalent (225)	No vaccine (826)	Not reported	9 women were hospitalised within 14 days of vaccination. 11 women were hospitalised for reasons that related to delivery. 2 were not related to delivery and 1 had influenza-like illness within 5 days of receipt of vaccine.
Nordin et al. (2013)[82]	Retrospective cohort	Trivalent (75906)	No vaccine (126246)	Not reported	Adverse event in the first 3 days after vaccination, adjusted incident rate ratio (IRR) 1.12, (95% CI: 0.81 to 1.55). Adverse event in days 1 to 42 post influenza vaccination, adjusted IRR 0.90 (95% CI: 0.68–1.19) New diagnosis of thrombocytopenia, adjusted IRR 0.90 (95% CI: 0.68 to 1.19). Acute neurologic event, adjusted IRR 0.92 (95% CI: 0.54 to 1.56). Among vaccinated women, there were no cases of Guillian-Barré syndrome, optic neuritis, Bell's palsy, or transverse myelitis. Adjusted for pre-existing high-risk conditions, receipt of care in the first trimester, and hospitalisation before the vaccination or index date.
Yeager et al. (1999)[104]	Prospective single arm study	Trivalent (319)	N/A	Split-virion, adjuvant not reported.	17 (5.3%) of women reported adverse reactions. All reactions were described as mild and consisted mostly of influenza-like symptoms (4.4%) and soreness at the injection site (0.9%). No other adverse events were noted, including premature birth.

Study	Design	Vaccination group (n)	Control group (n)	Adjuvant & formulation	Results
Zaman et al. (2008)[59]	Randomised controlled trial	Trivalent (172)	Pneumococcal vaccine (168)	Not reported	**Adverse events** Minor local and systemic reactions 13 (7.6%) Local pain 7 (4.1%) Fever within 72 hours 23 (13.4%)
Candela et al. (2012)[53]	Prospective single arm study	Monovalent H1N1 09 (370)	N/A	Subunit MF59-adjuvant	**Adverse events** 26 medically confirmed and 33 self-reported adverse events were reported. Systemic adverse events included common cold (11.5%), cough (11.5%), diarrhoea, and gastroenteritis (11.5%). **Adverse events of special interest** Nil **Serious adverse events** Nil suspected to be vaccine related were identified.
Cristiani et al. (2011)[54]	Prospective cohort	Monovalent H1N1 09 (3)	Monovalent H1N1 09 + trivalent (10)	Subunit MF59-adjuvant	**Adverse events** 11 in 7 pregnant women, Local adverse events; 4 injection site reactions. Systemic adverse events; 4 headaches, 2 fatigue, and 1 respiratory tract infection.
Hårmark et al. (2011)[86]	Prospective cohort	Monovalent H1N1 09 (72)	Vaccinated risk groups (3703)	Subunit MF59-adjuvant	Risk of adverse event following influenza vaccination, OR 2.61 (95% CI: 1.55 to 4.40). The multivariate logistic regression model compared gender, age (0–52.5 years), (52.5 to 61.9 years), (61.9 to 67.2 years), (67.2 to 90.0 years), cardiovascular disease, pulmonary disease, immunodeficiency, and pregnancy.
Horiya et al. (2011)[88]	Prospective cohort	Monovalent H1N1 09, 2 doses (128)	Monovalent H1N1 09, 1 dose (82)	Split-virion adjuvant free	**Adverse events** Redness at the vaccination site was the most common reaction, followed by local symptoms such as pain, and induration. Less common were systemic symptoms such as headache, malaise, fever, and nausea. **Serious adverse events** No serious adverse events requiring medical intervention were reported.

Study	Design	Vaccination group (n)	Control group (n)	Adjuvant & formulation	Results
Hwang et al. (2011)[90]	Prospective cohort study	Monovalent H1N1 09 (21)	Vaccinated risk groups (875)	Split-virion adjuvant free	Incidence of local reactions in pregnant women 38.1%. Incidence of systemic reactions in pregnant women 33.3%. Odds of reaction following vaccination for pregnant women Local adjusted OR 1.82 (95% CI: 0.72 to 4.56). Systemic, adjusted OR 0.95 (95% CI: 0.37 to 2.45). The multivariate logistic regression model compared age < 20, (20 to 29), (30 to 39), (40 to 49), >50, Body Mass Index (BMI), smoking, regular alcohol consumption, and comorbidity. Adjusted for smoking, regular alcohol use, and pregnancy.
Jackson et al. (2011)[56]	Randomised controlled trial	Monovalent H1N1 09 25μg HA (60)	Monovalent H1N1 09 49μg HA (60)	Split-virion adjuvant free	**Adverse events** Mild to moderate pain at injection site 25μg, 25%. 49μg, 35%. Erythema < 50mm, 25μg, 8%. 49μg, 13%. Swelling/induration, 25μg, 7%. 49μg, 2%. Fever (not defined), 25μg, 8%. 49 μg, 7%. Malaise, 25 μg, 31%. 49μg, 40%. Oral temperature >37.8, 25μg, 0%. 49μg, 2%. Headache 25μg, 28%. 49μg, 30%. **Serious adverse events** 18 serious adverse events were reported for 15 women; all were considered to be unrelated to the vaccine, the more common were 6 reports of postpartum haemorrhage, 2 reports of preterm contractions, and 2 reports of severe pre-eclampsia.
Lim et al. (2010)[92]	Prospective single arm survey	Monovalent H1N1 09 (190)	N/A	Split-virion adjuvant free	**Adverse events** Local adverse reactions; soreness (41.6%) and redness (8.4%) at the injection site. Systemic symptoms; fatigue (36.6%), myalgia (23.7%), dizziness (23.2%), headache (20%), fever (13.7%), chills (10%), respiratory symptoms including rhinorrhoea (14.2%), sore throat (11.1%), cough (8.4%), gastrointestinal symptoms including diarrhoea (5.8%), and abdominal pain (5.3%).

Study	Design	Vaccination group (n)	Control group (n)	Adjuvant & formulation	Results
Lin et al. (2012)[93]	Retrospective cohort	Monovalent H1N1 09 (202)	No vaccine (206)	Split-virion adjuvant free	**Adverse events** Systemic adverse events occurred in 4 women (2.0%). These were made up of fever, cough, runny nose, nasal congestion, and skin itching. **Serious adverse events** No serious adverse events were reported.
Mackenzie et al. (2012)[94]	Prospective cohort	Monovalent H1N1 09 (104)	No vaccine (13)	Not reported	**Serious adverse events** No serious adverse events were reported.
Omon et al. (2011)[97]	Prospective single arm	Monovalent H1N1 09 (651)	N/A	Split-virion adjuvant free	**Adverse events** Fever occurred in 11 (1.9%) women. Other adverse events during the 48-hour period following the vaccination included asthenia, headaches, pain at the site of injection, and influenza-like symptoms occurred in 11 (1.9%) women. 141 (25%) women reported adverse events during pregnancy and these included preterm labour, arterial hypertension, gestational diabetes, infections, and premature rupture of membranes. **Serious adverse events** 56 (9.6%) were hospitalised during pregnancy, mainly related to hypertension or preterm labour. Further investigation of each case was not reported.
Opperman et al. (2012)[77]	Prospective cohort	Monovalent H1N1 09 (323)	No vaccine (1329)	Split-virion adjuvant free or Split-virion AS03-adjuvant	**Adverse events** Systemic adverse events occurred in 16.2% of women vaccinated with non-adjuvanted split-virion vaccine. Systemic adverse events occurred in 25.6% of women vaccinated with AS03-adjuvanted vaccine. Local adverse events occurred in 38.9% and in 71.1% of women vaccinated with the non-adjuvanted and AS03-adjuvanted vaccines respectively.

Study	Design	Vaccination group (n)	Control group (n)	Adjuvant & formulation	Results
Taveres et al. (2011)[103]	Prospective single arm study	Monovalent H1N1 09 (267)	N/A	Split-virion, ASO3 adjuvant	**Medically attended adverse event** Within the 31 day post-vaccination period, 59 (22.1%). The most common were upper respiratory tract infections (3.8%) and urinary tract infections (3%). **Serious adverse event** During the 6-month follow-up period, 34 (12.7%). These were mostly associated with an adverse pregnancy outcome. i.e., spontaneous abortions (table 11) premature labour (table 9), and preeclampsia/hypertension (table 8). No adverse events of special interest reported.
Tsatsaris V, et al. (2011)[58]	Single arm prospective study	Monovalent H1N1 09 (107)	N/A	Split-virion adjuvant free	**Adverse events** Local adverse events; pain 20 (19%), induration 3 (3%), and erythema 2 (2%). Systemic reactions; asthenia 24 (22%), headache 10 (9%), myalgia 3 (3%), arthralgia 2 (2%), hyperhidrosis 2 (2%), pyrexia 1 (1%), and chills 2 (2%). **Serious adverse events** Serious adverse events were reported for 13 women. An independent committee considered none related to the vaccine. No adverse events of special interest were reported.
Deinard, Ogburn. (1981)[78]	Prospective cohort	Monovalent Hsw1N1 76 (176)	No vaccine (517)	Either split or whole virus vaccine. Adjuvant not reported.	**Adverse events** Systemic adverse events included arthralgia or myalgia 2.7%, headache 1.6%, fever 37.8-38.6 1.2%, fever >38.6 0.1%, malaise 1.1%, chills 0.6%, and cough 0.2%. **Serious adverse events** No major or life-threatening reactions occurred following vaccination.
Sumaya et al. (1979)[57]	Prospective cohort	Monovalent Hsw1N1 76 (56)	No vaccine (56)	Whole virus, adjuvant not reported	Systemic reactions; 3 fever, 1 each for dizziness, headache, influenza-like symptoms, and coryza.

Table 8 Preeclampsia in pregnant women following exposure to the influenza vaccine

Study	Design	Vaccination group (n)	Control group (n)	Adjuvant & formulation	Result
Conlin et al. (2013)[81]	Retrospective cohort	Monovalent H1N1 09 (10376)	Trivalent (7560)	Not reported	Preeclampsia or eclampsia 5.8% of women vaccinated with monovalent influenza A (H1N1) 09 vaccine. 5.2% of women vaccinated with seasonal trivalent vaccine.
Heikkinen et al. (2012)[87]	Mixed prospective and retrospective	Monovalent H1N1 09 (2295)	No vaccine (2213)	Subunit MF59-adjuvant	Adjusted OR 1.12 (95% CI: 0.81 to 1.55). Adjusted for parity, smoking, and maternal age.
Opperman (2012)[77]	Prospective cohort	Monovalent H1N1 09 (323)	No vaccine (1329)	Split-virion adjuvant free or Split-virion AS03-adjuvant	Adjusted OR 1.15 (95% CI: 0.54 to 2.46).†
Rubinstein (2013)[83]	Cross sectional study	Monovalent H1N1 09 (7293)	No vaccine (23195)	Subunit MF59-adjuvant	124 (1.7%) vaccinated 384 (1.7%) unvaccinated
Taveres et al. (2011)[103]	Prospective single arm study	Monovalent H1N1 09 (267)	N/A	Split-virion, ASO3 adjuvant	Preeclampsia/hypertension 4 (1.5%) within 19–30 days of vaccination.

† Propensity score adjusted, refer to Appendix V.

3.5 Foetal, birth, and infant outcomes

3.5.1 Premature birth (< 37 weeks)

Premature birth outcomes were reported in 22 studies in this review with a standard definition of less than 37 weeks gestation across all studies.[7, 18, 46, 53, 54, 77, 78, 80, 81, 83, 85, 87, 88, 91-93, 95-97, 101-103] There were 17 studies with outcomes of monovalent influenza A vaccines [18, 46, 53, 54, 77, 78, 80, 81, 83, 85, 87, 88, 91-93, 97, 103] and five for the trivalent seasonal vaccine.[7, 95, 96, 101, 102] Prospective study designs were performed in nine studies.[53, 54, 77, 78, 88, 92, 97, 102, 103] The remainder of studies were of retrospective[7, 18, 46, 80, 81, 83, 85, 91, 93, 95, 96, 101] or mixed retrospective/prospective design.[87] Timing of vaccination varied between the studies and the percentage of women vaccinated during their first trimester ranged from 1.2% to 39.7%.[46, 77, 78, 81, 83, 86-88, 91-93, 97, 101, 103] There were three with no women vaccinated during their first trimester[53, 54, 102] and four did not report what trimester vaccination took place.[7, 80, 85, 96]

Maternal risk factors that may confound estimates of rates or ratios of premature birth were described or analysed differently across the study designs. Studies that were using cohorts of healthy pregnant women had a reduced risk of confounding, however most did not report external risk factors such as smoking, alcohol use, or drug use[88, 92, 95, 102] with one exception.[87] The remainder of studies reported a variety of other variables including smoking,[18, 46, 77, 78, 83, 85, 87, 91, 93, 97] maternal weight,[46, 77, 83, 85, 91, 101] diabetes and other comorbidities,[18, 46, 77, 80, 97, 101, 103] prior preterm birth,[85, 93, 97] and alcohol or drug use.[46, 77, 83, 87, 93, 97] Eight of the studies statistically adjusted for one or more of the variables and they are described in more detail in Appendix V.[18, 46, 80, 85, 87, 91, 96] The remainder of studies presented data descriptively as frequencies and percentages.[53, 54, 77, 78, 81, 88, 92, 93, 95, 97, 101, 103]

Meta-analysis was performed for three retrospective cohort studies.[46, 85, 91] Each study contained large populations of singleton births in a country or large city, and were investigating outcomes of the influenza A (H1N1) 2009 vaccine.

The first study included in meta-analysis by Fell et al. conducted a retrospective cohort on Ontario residents in Canada.[85] All singleton hospital births of 20 weeks gestation or more with a birth weight equal to or greater than 500 g were included in the study. The groups consisted of 78% of pregnant women less than 35 years of age and 7.4% of the vaccinated women had a medical comorbidity.[85] Data was obtained from national databases on birthing outcomes, demographic characteristics, and socioeconomic status. Multivariable regression was used to calculate adjusted risk ratios and all adjusted models included maternal age, family income, and education. In all 23340 pregnant women were included in the vaccinated cohort and 32230 in the unvaccinated cohort. For the outcome of premature birth there was missing data from 60 in the vaccinated cohort and 139 of the unvaccinated cohort that was not included in the regression modelling. The trimester of vaccination could not be obtained and was not reported.[85] It was a well-conducted study meeting all eight of the relevant critical

appraisal criteria.

Kallen B, Olausson P. (2012) also included a whole of pregnant population based cohort, this time from Sweden.[91] Pregnant women who delivered after the 1st of October 2009 were compared to a group of pregnant women who delivered prior to that date. The highest recorded vaccination rate was in women aged 25 to 29 years with 80.2% of the vaccinated cohort being less than 35 years of age. All trimesters were vaccinated and 3165 (17%) were vaccinated in the first trimester.[91] In total 18844 infants were from vaccinated mothers and 84484 were in the group who delivered prior to the availability of the monovalent influenza vaccine.[91] Mantel–Haenszel odds ratio and approximate 95% confidence intervals were estimated and adjusted for adjusted for year of birth, maternal age, parity, smoking, BMI, preterm birth, low birth weight, and SGA<2 SD for age.[91] It was also a well-conducted study meeting all eight of the relevant critical appraisal criteria.

The final study included in the meta-analysis was a retrospective study that contained a nationwide cohort of pregnant women from Denmark.[46] Information was obtained from the medical birth register that has detailed records of all births in Denmark. Women were vaccinated in all trimesters, although only women vaccinated in their second and third trimester were included in meta-analysis as the small number of women (345) who were vaccinated in the first trimester were analysed separately.[46] In the vaccinated cohort 81% of pregnant women were less than 35 years of age.[46] The authors calculated a propensity score for each participant and then both cohorts were matched 1:1 and participants with no match excluded. Logistic regression was used to estimate prevalence odds ratios. Potential confounders that are included in the modelling are described in table 9. Potential confounders in propensity matched scores included maternal age, place of birth, degree of urbanisation, parity, smoking, pre-pregnancy body mass index, history of birth defects, preterm birth, spontaneous abortion, SGA, maternal comorbidities, drug use, health care utilisation, number of hospital admissions and hospital outpatient visits in the last 3 years, and number of drugs used in the last 6 months. It was a well-conducted study meeting all eight of the relevant critical appraisal criteria.

The meta-analysis of the three large population based cohorts investigating the safety of the influenza A (H1N1) 2009 vaccine was homogenous with an I^2 percentage of 0% and had a point estimate of 0.94 (95% CI: 0.89 to 0.99) as shown in figure 2.

The retrospective study by Hårberg et al. (2013) met the criteria needed to be included in meta-analysis, but could not be combined due to the use of hazard ratios.[18] Their study was also a whole of pregnant population who had singleton births in Norway and included pregnant women of whom 80% were aged less than 35 and 11% had a chronic illness. [18] The vaccinated cohort consisted of 25976 women and the unvaccinated 87335 pregnant women. [18] Data were obtained from national databases and the authors used a Cox proportional hazard model with gestational day as the underlying time metric

adjusting for age, parity, marital status, use of nutritional supplements during pregnancy, smoking during pregnancy, history of earlier foetal death, and eight chronic medical conditions. [18] It was a well-conducted study meeting all eight of the relevant critical appraisal criteria. They estimated the risk of premature birth following influenza (H1N1) 2009 vaccination as an adjusted HR of 1.00 (95% CI: 0.93 to 1.09), which is similar to the overall meta-analysis result and indicates that there is no adverse association between the monovalent influenza vaccine and premature birth in the large retrospective population studies.[18]

The seven prospective studies that reported results as a percentage had rates ranging from 1.2% to 9.9% for premature births of vaccinated pregnant women and they were all on the influenza A (H1N1) 2009 vaccine. [53, 54, 77, 78, 88, 92, 97, 103] The three remaining smaller retrospective studies that had an unvaccinated comparator group and calculated odds ratios had a point estimate that favoured the vaccinated cohort, with two indicating a statistically significant reduction,[80, 83] and one was not statistically significant.[87]

Seasonal influenza outcomes of premature birth were contained in five studies.[7, 95, 96, 101, 102] The study by Steinhoff et al (2012) that is a secondary analysis of the randomised control trial previously described by Zaman et al.[59] had small numbers and wide confidence intervals with a point estimate favouring the vaccinated cohort.[102] The studies by Black et al. (2004) and Sheffield et al (2012) had unmatched cohorts and no adjustment was made for their results on premature birth.[7, 101] Only Black et al. (2004) had a point estimate that favoured the unvaccinated cohort,[7] however the study also had the least comprehensive assessment of potential confounding factors and should be overlooked in preference to the studies of Munoz et al.[95] and Omer et al.[96] Munoz et al. (2005) did include assessment and matching of some potential confounders and had a point estimate that favoured the vaccinated cohort, although not statistically significant.[95]

Omer et al. (2011) conducted a retrospective cohort study in multiple sites in the USA.[96] Data were obtained from medical databases and the cohorts consisted of pregnant women vaccinated in unknown trimesters of pregnancy with 82.3% of the vaccinated cohort being under 35 years of age. They used primary adjusted models that identified covariates during the pre-influenza period. The authors conducted analysis of premature birth during periods of widespread influenza activity, and found a further reduction in the odds of giving birth to a premature infant following vaccination.[96]

The studies with the most comprehensive assessment of potential confounding factors and largest population wide cohorts indicated that there were no harmful effects of the vaccine on premature birth. The only study that conducted a separate analysis on first trimester vaccinations had a small but not statistically significant increase.[46] The methods of each study are described in more detail in Appendix V and the results, type of vaccine, and sample size are also described in table 9.

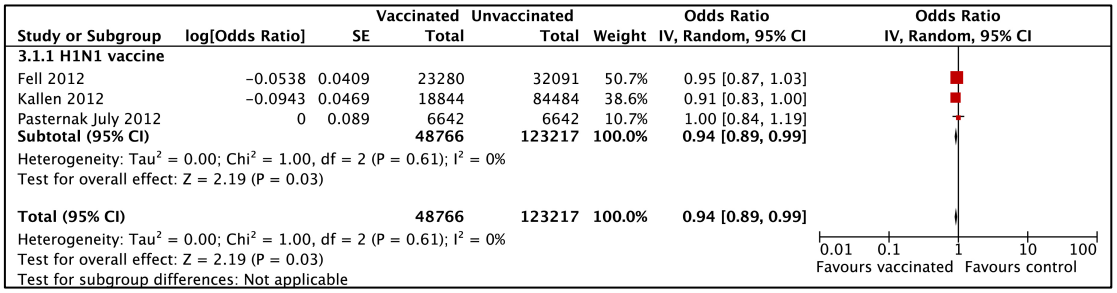

Figure 2 Meta-analysis of premature birth (< 37 weeks). Infants up to 6 months of vaccinated versus non-vaccinated pregnant women

Table 9 Premature birth (<37 weeks) in pregnant women following exposure to the influenza vaccine. Studies not included in meta-analysis.

Study	Design	Maternal age†	Trimester vaccinated	Vaccination group (n)	Control group (n)	Results
Black et al. (2004)[7]	Retrospective cohort	Not reported.	Not reported	Trivalent (3652)	No vaccine (44987)	7.37% Vaccinated 6.72% Unvaccinated (p=0.136).
Munoz et al. (2005)[95]	Retrospective cohort	Mean 30.7 and 30.8	2nd or 3rd trimester	Trivalent (225)	No vaccine (826)	5.5% vaccinated women 8% unvaccinated women (p=0.28). Unadjusted OR 0.67 (95% CI: 0.32 to 1.32)
Omer, et al. (2011)[96]	Retrospective cohort study	82.3% < 35 years	Not reported	Trivalent (578)	No vaccine (3590)	Putative influenza season adjusted OR 0.60 (95% CI: 0.38 to 0.94, p=0.02) Period of widespread influenza activity adjusted OR 0.28 (95% CI: 0.11 to 0.74, p=0.01). Adjusted for gestational age at first antenatal visit, maternal diabetes, multivitamin use in pregnancy, history of alcohol use during pregnancy, education less than 12th grade, and mother married.
Sheffield et al. (2012)[101]	Retrospective cohort	89% < 35 and 91% < 35 years	All, 439 (5%) in 1st trimester	Trivalent (8864)	No vaccine (76919)	460 (5%) vaccinated, 4612 (6%) unvaccinated (p=0.004)
Steinhoff et al. (2012)[102]	Randomised controlled trial	Mean 25.1 (range 18.0 to 36.0) and 24.9 (range 18.0 to 36.0)	3rd trimester	Trivalent (169)	Pneumococcal vaccine (167)	During circulating virus adjusted OR 0.32 (95% CI: 0.05 to 2.29). Adjusted for interval from immunisation to delivery.
Candela et al. (2012)[53]	Prospective single arm	Not reported.	2nd or 3rd trimester	Monovalent H1N1 09 (70)	N/A	4.3% of women vaccinated gave birth prematurely.

Study	Design	Maternal age†	Trimester vaccinated	Vaccination group (n)	Control group (n)	Results
Conlin et al. (2013)[81]	Retrospective cohort	92.5% < 35 and 92.7% < 35 years	All, 39.7% and 36.3% in 1st trimester.	Monovalent H1N1 09 (9435)	Trivalent (6759)	6.5% in (H1N1) 09 vaccine-exposed group 6.2% in trivalent seasonal influenza vaccine
Cristiani et al. (2011)[54]	Prospective cohort	Median 33 (range 25 to 39 years).	2nd or 3rd trimester	Monovalent H1N1 09 (3)	Monovalent H1N1 09 + trivalent (10)	1 preterm delivery (35 weeks of gestation)
Hårberg et al. (2013)[18]	Retrospective cohort	80% aged < 35 years	All, 2431 (9.4%) in the 1st trimester	Monovalent H1N1 09 (25976)	No vaccine (87335)	Adjusted HR 1.00 (95% CI: 0.93 to 1.09) Vaccination status and pandemic exposure were adjusted for each other and also adjusted for age, parity, marital status, use of nutritional supplements during pregnancy, smoking during pregnancy, history of earlier foetal death, and eight chronic medical conditions.
Heikkinen et al. (2012)[87]	Mixed prospective retrospective	Mean 31.6	All, 94 (4%) in the 1st trimester	Monovalent H1N1 09 (2295)	No vaccine (2213)	Adjusted OR 0.75 (95% CI: 0.55 to 1.01), adjusted HR 0.69 (95% CI: 0.51 to 0.92) Adjusted for parity, smoking, and maternal age.
Horiya et al. (2011)[88]	Prospective cohort	Mean 34.8 (SD±4.1) and 35.7 (SD±3.6)	All, 15 (7.1%) in the 1st trimester	Monovalent H1N1 09, 2 doses (128)	Monovalent H1N1 09, 1 dose (82)	7% vaccinated in the first or third trimester. 2% vaccinated in the second trimester. 4.2% all trimesters.
Lim et al. (2010)[116]	Prospective single arm	Mean 31.3 (SD±3.8)	All, 2 (1.2%) in the 1st trimester	Monovalent H1N1 09 (167)	N/A	Two babies (1.2%) were delivered preterm at the 35th gestational week; 42 babies (25.9%) were delivered between 36 and 38 weeks' gestation.

Study	Design	Maternal age[†]	Trimester vaccinated	Vaccination group (n)	Control group (n)	Results
Lin et al. (2012)[93]	Retrospective cohort	Mean 32.4 (SD±4.0) and 32.8 (SD±3.9)	All, 10 (4.9%) in the 1st trimester	Monovalent H1N1 (202)	No vaccine (206)	12 (5.9%) vaccinated 18 (8.7%) unvaccinated
Omon et al. (2011)[97]	Prospective single arm	Mean 31 (SD±4)	All, 29 (4%) < 15 weeks	Monovalent H1N1 09 (651)	N/A	7.2% of women vaccinated
Opperman et al. (2012)[77]	Prospective cohort	Median 33 and 32	All, 55 (17%) in the 1st trimester	Monovalent H1N1 09 (323)	No vaccine (1329)	29 (9.09%) vaccinated 122 (10.25%) unvaccinated
Pasternak et al. (2012)[46]	Retrospective cohort study	Mean 30.7 (SD±5.2) and 30.1 (SD±5.0)	First trimester only 345[‡]	Monovalent H1N1 09 330[§]	No vaccine 330[§]	Propensity matched odds ratio (POR) 1.32 (95% CI: 0.76 to 2.31).[¶]
Richards et al. (2013)[80]	Retrospective cohort	Mean 31.2 (SD±5.6)	Not reported	Monovalent H1N1 09 + trivalent (1125)	No vaccine (1581)	Adjusted OR, 0.63 (95% CI: 0.47 to 0.84) Adjusted for: maternal age, asthma, gestational diabetes, cardiovascular disease, hypertension during pregnancy, multiple birth, any pregnancy complication, any antiviral use, and site.

Study	Design	Maternal age[†]	Trimester vaccinated	Vaccination group (n)	Control group (n)	Results
Rubinstein et al. (2013)[83]	Cross sectional study	88.5% < 35 and 86.9% < 35 years	All, 2874 (39.4%) in first trimester	Monovalent H1N1 09 (7293)	No vaccine (23195)	Adjusted OR 0.79 (95% CI: 0.69 to 0.90) Adjusted antenatal visits, level of education, maternal age, income, parity, smoking, and history of pregnancy induced hypertension.
Taveres et al. (2011)[103]	Prospective single arm study	Mean 30.9	All, 42 (15.7%) in first trimester	Monovalent H1N1 09 (267)	N/A	5.4% of women vaccinated gave birth prematurely.
Deinard, Ogburn (1981)[78]	Prospective cohort	Mean 21.97 (SD$\pm$4.6)	All, 41 (23%) in first trimester	Monovalent Hsw1N1 76 (176)	No vaccine (517)	16 (9.1%) vaccinated 55 (10.6%) unvaccinated

[†] Maternal age is in years. The first listed are the vaccination group, and the second the control group. If only one is present that represents the entire study population.
[‡] Separate analysis was performed for infants with mothers who were vaccinated during their 1st trimester. The POR for 2nd and 3rd trimester is used in the meta-analysis.
[§] Propensity matched cohort sizes.
¶ Propensity score matched cohorts, refer to Appendix V

3.5.2 Very premature birth (<32 weeks)

Very premature birth outcomes were reported in five studies,[46, 85, 97, 101, 103] and four investigated outcomes for the influenza A (H1N1) 2009 vaccine.[46, 85, 97, 103] Two of these were retrospective studies that met the criteria outlined for meta-analysis.[46, 85] The analysis had a point estimate that favoured the vaccinated cohort, with the upper 95% confidence interval marginally crossing the null value and it was homogenous with an I^2 percentage of 19% as seen in figure 3. The results from Pasternak et al. include second and third trimester vaccination analysis only. Very premature birth for women vaccinated during their first trimester had an estimated OR 0.97 (95% CI: 0.63 to 1.53). Two remaining studies were single arm prospective studies and reported 0.8% and 1.1% of women in their vaccinated cohort having a very premature delivery.[97, 103]

Only one study reported outcomes for very premature birth with the trivalent seasonal vaccine.[101] The retrospective cohort study was conducted in hospitals in Texas USA over five influenza seasons between October 2003 and March 2008 and included 8690 women who received seasonal influenza vaccine, with 439 during the first trimester.[101] The vaccinated cohort had 89% of women who were under 35 years of age and the unvaccinated cohort was 91%. The vaccinated cohort had statistically significant differences with a history of obstetric complication, with 72% compared to 48% and diabetes 12% to 6%.[101] The unexposed cohort received prenatal care over the same period. The study reported that 0.7% of vaccinated women and 1.3% of the vaccinated cohort ($p<0.001$) had very premature births.[101] No adjustments or matching was performed on the study groups.

Overall there was no clinically or statistically significant adverse effect of the vaccine on very premature births.

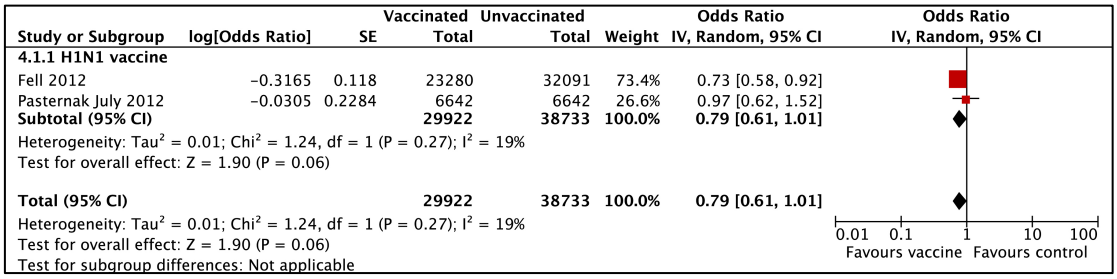

Figure 3 Meta-analysis of very premature birth (< 32 weeks). Vaccinated versus unvaccinated pregnant women with influenza A (H1N1) 2009

3.5.3 Foetal death

Foetal death outcomes were reported in 17 studies in this review[18, 77, 78, 81, 83, 85, 87, 88, 91, 93, 94, 97, 98, 100-103] and contained two new studies not discussed in previous sections.[98, 100] Trivalent seasonal influenza was only investigated in two studies[101, 102] and the remainder investigated the monovalent influenza A (H1N1) 2009[18, 77, 81, 83, 85, 87, 88, 91, 93, 94, 97, 98, 100, 103] and (Hsw1N1) 1976 vaccine.[78] Foetal death following influenza vaccination during pregnancy had a variety of definitions that complicated comparison between studies and they are described in table 10.

Two studies were specifically designed to investigate the risk of foetal death following influenza A (H1N1) 2009 vaccine.[101, 102] Sammon et al. (2012)[100] conducted a retrospective cohort study during the 2009 to 2010 influenza A (H1N1) vaccination campaign in the UK. Data were obtained from the UK General Practice Research Database that contains primary care records for 8.4% of the UK population and an algorithm was used to identify pregnancies and estimate start and end dates. Pregnant women with at least 6 months of data available prior to their last menstrual period date were included. The vaccinated cohort consisted of 9445 pregnant women with an unvaccinated cohort of 26993,[100] and had a mean age 29.9 years for those that delivered an infant, with 5.9% of pregnant women included also having another clinical risk for influenza.[100] The authors conducted a discrete survival analysis with separate hazard ratios for weeks 9 to 12, weeks 13 to 24, and weeks 25 to 42. Only weeks 25 to 42 are included in the foetal death analysis of this review. They used two models, with one assessing for an adverse association and one for a protective effect. Potential confounders that were investigated in the analysis included maternal age, history of spontaneous loss, diabetes, pre-pregnancy smoking status, pre-pregnancy alcohol use, pre-pregnancy body mass index, the number of consultations in the 6 months before the last menstrual period, and being in an influenza risk group. None of these potential confounders changed the point estimate of the HR for vaccination by more than 10% and they were not included in the final models.[100]

Foetal death following influenza A (H1N1) 2009 vaccination was also the sole outcome of interest for the study by Pasternak et al. (2012).[98] Using the nationwide registry they conducted a retrospective cohort study of all singleton pregnancies in Denmark including live births, still births, and pregnancies with an abortive outcome. Pregnant women included in the cohorts had a mean age of 30 (SD±5.2) (n=7062) in the vaccinated cohort and 30.9 (SD±4.7) (n=47525) in the unvaccinated cohort.[98] The study included a period from November 2009 to 30 September 2010 and coincided with the start of the vaccination campaign. A detailed assessment of comorbidities was performed and these were used in propensity modelling. Cox proportional hazards regression with gestational age in days as the underlying time scale was used to assess foetal death, stillbirth, and spontaneous abortion. Logistic regression was used to estimate each woman's probability to be vaccinated conditional on covariates.

Women were excluded with a non-overlapping probability of being vaccinated at the extreme ends of score distribution.[98] Multiple variables were included in the propensity models and these are described in more detail in table 10. Both studies met all the appraisal criteria and were well-conducted retrospective cohort studies.

Studies for trivalent seasonal influenza vaccine included a randomised controlled trial that has a small sample size and not large enough to accurately assess this outcome,[102] and a retrospective cohort that indicated a reduction in the number of episodes of foetal death following vaccination that was statistically significant.[101] The retrospective cohort study reported unadjusted rates and had unmatched cohorts.

Studies on the influenza A (H1N1) 2009 vaccine included two retrospective cohort studies that had a point estimate favouring the vaccinated cohort, with the upper confidence interval crossing the null value.[18, 91] Both were well conducted studies, although one did not have a clear definition of foetal death.[91] There were two retrospective cohort studies that also had a point estimate which favoured the vaccinated cohort with an upper confidence interval that did not cross the null value and indicated the results were statistically significant.[85, 98]

The study by Sammon et al. had a hazard ratio point estimate that favoured the unvaccinated cohort with relatively wide confidence intervals that did not indicate a statistically significant result. One mixed prospective and retrospective case study had a point estimate that favoured the unvaccinated cohort with wide non-statistically significant confidence intervals due to only three cases in the vaccinated and two in the unvaccinated cohort.[87]

There were eight studies that had a description, rate or percentage of foetal death that occurred in their studies. Two prospective cohort studies[77, 94] and a prospective single arm study[103] reported no foetal deaths. A cross sectional study reported a lower percentage for the vaccinated cohort with a non-statistically significant reduction following a chi-square test.[83] A retrospective study that had a comparator group of the trivalent seasonal vaccine reported similar percentages for both the (H1N1) 2009 cohort and the trivalent cohort.[81]

The remaining studies that included a prospective single arm and cohort study, one retrospective cohort study, and the prospective study on the (Hsw1N1) 1976 vaccine had very small numbers and provide limited additional insight into this outcome.[78, 88, 93, 97]

The outcome of foetal death had no statistically significant increase following vaccination during pregnancy, although one study that used survival analysis had a point estimate that favoured the unvaccinated cohort with wide non-statistically significant 95% confidence intervals.[100] Some studies indicated that the vaccine could potentially decrease the risk of foetal death,[18, 91] however large prospective studies are needed to confirm these findings. There was clinical and methodological

diversity in the cohort studies and meta-analysis was unable to be performed. Further description of the studies can be found in table 10 and Appendix V

Table 10 Foetal death following exposure of pregnant women to the influenza vaccine

Study	Design	Vaccination group (n)	Control group (n)	Results	Definitions
Sheffield et al. (2012)[101]	Retrospective cohort	Trivalent (8864)	No vaccine (76919)	30 (0.3%) vaccinated 436 (0.6%) unvaccinated (p=0.006)	Foetal death with weight ≥ 500g.
Steinhoff et al. (2012)[102]	Randomised controlled trial	Trivalent (169)	Pneumococcal vaccine (167)	3 stillbirths in influenza vaccinated cohort. Nil in the pneumococcal vaccinated cohort.	No definition described. It is unclear what gestational ages were included.
Conlin et al. (2013)[81]	Retrospective cohort	Monovalent H1N1 09 (9435)	Trivalent (6759)	6.4% monovalent (H1N1) 2009 vaccine 6.5% trivalent seasonal vaccine experienced	ICD-9 codes. Ectopic or molar pregnancy, other pregnancy with abortive outcome, intrauterine death, outcome of delivery V27 (1,3,4,6,7), and procedure indicating foetal death. Gestational weeks not clear.
Fell et al. (2012)[85]	Retrospective cohort	Monovalent H1N1 09 (21363) + trivalent (1977)	No vaccine (32230)	Adjusted RR 0.66 (95% CI: 0.47 to 0.91) Adjusted for maternal age, family income, education, and maternal smoking.	Intrauterine death > 20 weeks gestation.

Study	Design	Vaccination group (n)	Control group (n)	Results	Definitions
Hårberg et al. (2013)[18]	Retrospective cohort	Monovalent H1N1 09 (25976)	No vaccine (87335)	78 foetal deaths in vaccinated cohort, HR 0.88 (95% CI: 0.66 to 1.17). Vaccination status and pandemic exposure were adjusted for each other and also adjusted for age, parity, marital status, use of nutritional supplements during pregnancy, smoking during pregnancy, history of earlier foetal death, and eight chronic medical conditions.	Miscarriage or stillbirth > 12 weeks gestation.
Heikkinen et al. (2012)[87]	Mixed prospective retrospective	Monovalent H1N1 09 (2295)	No vaccine (2213)	Adjusted OR 1.44 (95% CI: 0.23 to 8.90) Adjusted for parity, smoking, and maternal age.	Foetal death > 22 weeks gestation.
Horiya et al. (2011)[88]	Prospective cohort	Monovalent H1N1 09, 2 doses (128)	Monovalent H1N1 09, 1 dose (82)	One aborted a 5-month twin gestation 40 days after immunisation.	No definition described. It is unclear what gestational ages were included.
Kallen and Olausson (2012)[91]	Retrospective cohort	Monovalent H1N1 (18 844)	No vaccine (84 484)	Adjusted OR 0.81 (95% CI: 0.59 to 1.12). Adjusted for year of birth, maternal age, parity, smoking, BMI, preterm birth, low birth weight, and SGA<2 SD for age.	No definition described. It is unclear what gestational ages were included.
Lin et al. (2012)[93]	Retrospective cohort	Monovalent H1N1 (202)	No vaccine (206)	Nil in vaccinated group 1 in unvaccinated group.	No definition described. It is unclear what gestational ages were included.
Mackenzie et al. (2012)[94]	Prospective cohort	Monovalent H1N1 09 (104)	No vaccine (13)	No reported stillbirths	No definition described. It is unclear what gestational ages were included.

Study	Design	Vaccination group (n)	Control group (n)	Results	Definitions
Omon et al. (2011)[97]	Prospective single arm study	Monovalent H1N1 09 (651)	N/A	1 foetal death (0.2%)	No definition described. It is unclear what gestational ages were included.
Opperman et al. (2012)[77]	Prospective cohort	Monovalent H1N1 09 (323)	No vaccine (1329)	Nil foetal deaths in either cohort	No definition described. It is unclear what gestational ages were included.
Pasternak et al. (2012)[98]	Retrospective cohort	Monovalent H1N1 09 (7062)	No vaccine (47524)	Adjusted HR 0.44, (95% CI: 0.20 to 0.94).†	Foetal death > 22 weeks gestation.
Rubinstein et al. (2013)[83]	Cross sectional study	Monovalent H1N1 09 (7293)	No vaccine (23195)	25 (0.3%) vaccinated 111 (0.5%) unvaccinated (p=0.08)	Intrauterine death of the foetus in a pregnancy > 22 weeks.
Sammon et al. (2012)[100]	Retrospective cohort	Monovalent H1N1 09 (9445)	No vaccine (26993)	HR 0.70 (95% CI: 0.47 to 1.03) immunity model. HR 1.56 (95% CI: 0.73 to 3.34) toxicity model.	Foetal death in gestational weeks 25 to 43
Taveres et al. (2011)[103]	Prospective single arm study	Monovalent H1N1 09 (267)	N/A	Nil stillbirths	Foetal loss > 24 weeks gestation.
Deinard, Ogburn (1981)[78]	Prospective cohort	Monovalent Hsw1N1 76 (176)	No vaccine (517)	1 in each cohort.	Foetal death > 20 weeks gestation.

† Propensity score modelling performed, refer to Appendix V.

3.5.4 Spontaneous abortion

Spontaneous abortion outcomes were reported in 8 studies in this review.[77-79, 87, 94, 98, 100, 103] Only one was investigating the trivalent seasonal vaccine,[79] and the remainder were investigating the monovalent influenza A (H1N1) 2009[77, 87, 94, 98, 100, 103] or (Hsw1N1) 1976 vaccine.[78] The gestational age of 'spontaneous abortion' was inconsistent in the studies included and two studies did not have a clear definition.[77, 94] Spontaneous abortion is the most common complication of early pregnancy,[117] but despite this the numbers receiving the vaccine prior to the period used to define spontaneous abortion are low and that has resulted in smaller sample sizes in this outcome.

Only one case control study included spontaneous abortion outcomes for the seasonal trivalent influenza vaccine.[79] The study was conducted in the USA amongst health sites linked by a data network and was conducted over two influenza seasons between 2005 and 2007, involving a cohort of pregnant women aged 18 to 44 at the time of pregnancy loss.[79] The mean age in the cohort with pregnancy loss prior to 16 weeks was 31.7 years (SD±6.0) and in the group with no pregnancy loss 29.3 years (SD±5.4), with 16.5% of all pregnant women also having a chronic medical condition. Pregnancy losses through to 16 weeks were included to capture events in the weeks following vaccine exposure. Controls were randomly selected and matched based on the closest last menstrual period, as well as having confirmed pregnancy and delivery beyond 20 weeks. Logistic regression was performed for maternal age, parity, maternal diabetes, and health care utilisation. McNemar tests were used for dichotomous variables. The results had an odds ratio point estimate that favoured the vaccinated group in the 28-day exposure window following vaccination,[79] although the confidence interval indicates that this result was not statistically significant. There were limitations including possible vaccinated women in the control cohort, and some potential confounders that increase the risk of spontaneous abortion were not included such as ethnicity, alcohol, and other medical conditions. The other issue that is common with most of the retrospective studies is that spontaneous abortion needed to be identified in a medical setting and this may not always be the case in an early spontaneous abortion. In this particular study spontaneous abortions prior to 5 weeks of gestation were excluded.

Pasternak et al.'s (2012) retrospective cohort study on all live births in Denmark investigated the association of the influenza A (H1N1) 2009 vaccine with spontaneous birth up to 22 weeks and has been described in the previous section.[98] Spontaneous abortion only consisted of pregnant women vaccinated during their first trimester and had a hazard ratio point estimate that favoured the unvaccinated control cohort. Although the confidence interval crossed the null value and was non-statistically significant,[98] adverse effects on spontaneous abortion cannot be ruled out from this study. Whilst there was a comprehensive number of potential confounding factors included in their analysis, not all potential confounding factors were able to be included such as some comorbidities, alcohol, and

non-prescription drug use.

The other study that was specifically designed to assess the outcomes of foetal death and spontaneous abortion following influenza A (H1N1) 2009 vaccination is also described in the previous section.[100] Both models for foetal death between the weeks (9 to 12) and (13 to 24) calculated hazard ratios with a point estimate that favoured the vaccinated cohort with a confidence interval suggesting that the results were statistically significant. Despite this, their results also indicated that the vaccine resulted in a reduction in foetal death at a time when the influenza virus was in limited circulation and no such association should exist. The authors concluded there was residual confounding that was unable to be measured.[100]

A prospective single arm study described the number of pregnant women who experienced foetal loss prior to 24 weeks and four occurred during their study. The remaining studies had low numbers, or unclear definitions of gestational age, or did not specify the number of women vaccinated prior to the event of interest and provide limited additional insight into this outcome.[77, 78, 87, 94]

Whilst these studies show no statistically significant increase in spontaneous abortion, even a very small increase due to a vaccine would be of great clinical significance. Both studies by Irving et al. (2013) and Pasternak et al. (2012) discuss the preliminary nature of their first trimester findings.[79, 98] The studies were clinically and methodologically diverse and meta-analysis was unable to be performed. Results are described in further detail in table 11.

Table 11 Spontaneous abortion following exposure of pregnant women to the influenza vaccine

Study	Design	Trimester vaccinated	Study group (n)	Control group (n)	Results	Definitions
Irving et al. (2013)[79]	Case control study	31 in the 1st trimester.	Foetal loss ≤ 16 weeks gestation ± trivalent influenza vaccination. (n=243)	No foetal loss ≤ 16 weeks gestation ± trivalent influenza vaccination.(n=243)	Receipt of the influenza vaccine in the 28 days prior to spontaneous abortion, adjusted OR 1.23 (95% CI: 0.53 to 2.89, p=0.63) Adjusted for maternal age, parity, maternal diabetes, and health care utilisation.	Pregnancy loss ≤ 16 weeks of gestational age.
Heikkinen et al. (2012)[87]	Mixed prospective retrospective	94 in the 1st trimester.	Monovalent H1N1 09 (Unclear)	No vaccine (2213)	Nil in vaccinated cohort. 9 cases in unvaccinated cohort.	Foetal death < 22 weeks gestation.
Mackenzie et al. (2012)[94]	Prospective cohort	19 in the 1st trimester	Monovalent H1N1 09 (Unclear)	No vaccine (13)	4 spontaneous miscarriages reported by 3 women.	No definition described. It is unclear what gestational ages were included.
Opperman et al. (2012)[77]	Prospective cohort	55 in the 1st trimester	Monovalent H1N1 09 (Unclear)	No vaccine (1329)	Adjusted HR 0.89 (95% CI: 0.36 to 2.19). Adjusted for vaccination time and study entry.	No definition described. It is unclear what gestational ages were included.
Pasternak et al. (2012)[98]	Retrospective cohort	2736 in the 1st trimester	Monovalent H1N1 09 (2736)	No vaccine (32627)	Adjusted HR 1.11 (95% CI: 0.71 to 1.73).†	Foetal death between 7 and 22 weeks gestation.

Study	Design	Trimester vaccinated	Study group (n)	Control group (n)	Results	Definitions
Sammon et al. (2012)[100]	Retrospective cohort	4912 vaccinated weeks in the 1st trimester, 4695 in the 2nd trimester‡	Monovalent H1N1 09 (N/A) §	No vaccine (26993)	Foetal death in weeks 9 to12, HR 0.74 (95% CI: 0.62 to 0.88) immunity model. HR 0.56 (95% CI: 0.43 to 0.73) toxicity model. Foetal death in weeks 13 to 24, HR 0.59 (95% CI: 0.45 to 0.77) immunity model. HR 0.45 (95% CI: 0.28 to 0.73) toxicity model.	Foetal loss at any time between the 9th week of pregnancy and delivery.
Taveres et al. (2011)[103]	Prospective single arm study	42 in 1st trimester, 120 prior to 24 weeks	Monovalent H1N1 09 (120)	N/A	4 spontaneous abortions occurred (3.3%).	Foetal death < 24 weeks gestation.
Deinhard, Ogburn (1981)[78]	Prospective cohort	41 in 1st trimester.	Monovalent Hsw1N1 76 (Unclear)	No vaccine (517)	2 women had a spontaneous abortion following vaccination in the first trimester. 6 women in the unvaccinated group had a spontaneous abortion.	Foetal death < 20 weeks gestation.

† Propensity matched cohorts, refer to Appendix V.
‡ Presented as weeks at risk following vaccination per trimester.
§ Crude numbers not available; refer to vaccinated weeks per trimester.

3.4.5 Congenital abnormality

Congenital abnormality outcomes were reported in 15 studies.[46, 57, 77, 78, 81, 83, 87-89, 91, 94, 95, 97, 101, 103] The studies with outcomes of interest contained three investigating trivalent seasonal vaccines,[89, 95, 101] 10 on monovalent influenza A (H1N1) 2009, [46, 77, 81, 83, 87, 88, 91, 94, 97, 103] and two on (Hsw1N1) 1976 vaccine.[57, 78]

The developing foetus is at most risk during the first trimester,[52] so the timing of the vaccination in relation to the date of conception is an important factor. Not all studies included a sub group analysis of women vaccinated during their first trimester, or restricted their results to this population. The definition used for congenital malformation was not standard amongst the studies as some of the studies included minor malformations and major malformations, whilst others just counted major malformations. The majority of studies used European Surveillance of Congenital Anomalies (EUROCAT) guidelines[63], or Centre for Disease Control Metropolitan Atlanta Congenital Defects Program (CDC MACDP) guidelines,[64] or the Swedish Birth Defects Register, and the Medical Birth Registry for classification of congenital abnormalities. Other studies took the information directly from medical discharge summaries or medical records, and undertook deliberation by a panel of clinicians to classify congenital malformations. The methods of classification are described for each study in table 12.

Some maternal risks have been described previously that may increase the risk of congenital abnormalities. Other than age, the overall assessment of potential confounding variables was not performed well. Only one study included folic acid supplementation in its assessment of participant characteristics,[97] six assessed level of smoking,[46, 78, 83, 87, 91, 97] four assessed maternal weight,[46, 83, 91, 101] five assessed diabetes and other comorbidities,[46, 97, 101, 103] and four assessed alcohol or drug use.[46, 83, 87, 97] Congenital abnormalities due to some chromosomal conditions, genetic disorders, and other known causes such as infection were also managed differently in the studies. These are explained in more detail in the summary of study outcomes.

There were three studies on a trivalent seasonal influenza vaccine[89, 95, 101] and two reported no congenital malformations in the vaccinated cohorts.[89, 95] The study by Sheffield et al. (2012) investigating major malformations had a point estimate odds ratio marginally over the null value and precise confidence intervals.[101] They were able to perform a sub-group analysis on women vaccinated during their first trimester that had a point estimate favouring the vaccinated cohort and non-statistically significant confidence intervals.[101]

There were four studies investigating the influenza A (H1N1) 2009 vaccine that had an unvaccinated comparator group and calculated odds ratios.[46, 77, 87, 91] A retrospective nation-wide cohort study by Pasternak et al. (2012) investigating major birth malformations used EUROCAT definitions for women vaccinated in their first trimester. They excluded infants with chromosomal defects, genetic disorders,

defect syndromes with known causes, and congenital viral infections possibly associated with birth defect e.g. rubella. They had an odds ratio point estimate that favoured the unvaccinated control cohort, but with only 33 cases and matched cohorts of 330 pregnant women each, it had wide confidence intervals with a high upper limit.[46] In the study the population exposed to the vaccine during the first trimester was women with comorbidities, which were a potentially higher risk population for congenital malformations than the unvaccinated cohort.

Opperman et al.'s (2012) prospective cohort investigating major malformations contained 20 participants who were vaccinated within four weeks prior to conception, as well as during the first trimester.[77] Their cohorts excluded women with exposure to teratogenic or fetotoxic substances including multiple prescription medications, exposure to antiviral medication, treatment for influenza, malignant tumours, and any convulsions including febrile convulsion. Results indicated an odds ratio point estimate favouring the vaccinated cohort for 'all malformations' using EUROCAT definitions.[77] They also did a sub group analysis of those women vaccinated during their first trimester only and the point estimate indicated null effect, with relatively wide confidence intervals due to the smaller number of women vaccinated.[77]

Kallen and Olausson's (2012) retrospective cohort used Swedish Medical Birth Register definitions which excluded less significant malformations and had an odds ratio point estimate 0.1 above the null value for pregnant women vaccinated in their first trimester, with precise confidence intervals.[91] The authors also performed a separate analysis for women vaccinated during weeks one to nine gestation and this estimate also was close to the null value, with precise confidence intervals.[91]

Heikkinen et al.'s (2012) mixed prospective and retrospective cohort study had each potential congenital malformation adjudicated by an expert panel that was blinded to the vaccination status of the infant's mother. Malformations were retained if they were listed on the EUROCAT guidelines. Malformations diagnosed prior to vaccination and chromosomal malformations were excluded. Pregnant women vaccinated during any trimester had an odds ratio point estimate that favoured the unvaccinated control cohort, with non-statistically significant confidence intervals.[87]

The remaining studies on the influenza A (H1N1) vaccine contained descriptive data and had rates of major congenital malformation that ranged between 0% and 4.7%.[81, 83, 88, 103] These studies included all congenital abnormalities regardless of possible cause.[81, 83, 88, 103] The two remaining studies that investigated the monovalent (Hsw1N1) 1976 vaccine had descriptive results on all congenital malformations and one performed a chi-square analysis that estimated a significant reduction of congenital abnormalities in the vaccinated cohort.[57, 78] All results are described in more detail in table 12.

Meta-analysis was unable to be performed due to the diversity in definitions and methodology. Similar to the outcome of spontaneous abortion, even a small increase in harm in this outcome would likely

outweigh the benefit of being vaccinated during the first trimester for healthy women, when the risk from complication from influenza is at its smallest.[5, 12] No studies indicated a statistically significant increase in congenital malformation following influenza vaccination. Descriptive studies that reported percentages are difficult to align with external congenital malformation rates from surveillance or other studies, as there are many variables and differences in methods that need to be taken into consideration. Four studies were able to compare vaccinated and unvaccinated cohorts during the first trimester when the foetus is at most risk.[46, 77, 91, 101] Studies by Pasternak et al (2012) and Opperman et al (2012) had upper odds ratio confidence intervals at or above 2 and it is not possible to conclude with certainty that the vaccine had no adverse effect on congenital malformations in these studies.[46, 77] The studies by Kallen and Olausson., (2012) and Sheffield (2012) had more statistical power and confidence intervals are closer to the null value, indicating that an association with the influenza vaccination and congenital malformation is unlikely in these studies.[91, 101] Clinically there is no evidence that the vaccine is unsafe to use in the first trimester. However, the evidence would be strengthened with increased sample size of the vaccinated cohort. Comprehensive assessment and adjustment or matching of potential variables that may contribute to premature birth is also required.

Table 12 Congenital malformation following exposure of pregnant women to the influenza vaccine

Study	Design	1st trimester vaccinations	Vaccination group (n)	Control group (n)	Results	Definitions
Hulka (1964)[89]	Pseudo-randomised controlled trial	13	Seasonal (13)	Placebo (181)	No congenital anomalies in pregnant women vaccinated in their first trimester.	No definition described.
Sheffield et al. (2012)[101]	Retrospective cohort	439 (5%)	Trivalent (8864)	No vaccine (76919)	1st trimester unadjusted OR 0.67 (95% CI: 0.36 to 1.26). 2nd and 3rd trimester, unadjusted OR 1.01 (95% CI: 0.85 to 1.21).	Major malformations, significant functional or cosmetic impairment or those that were life limiting.
Munoz et al. (2005)[95]	Retrospective cohort	Nil	Trivalent (225)	No vaccine (826)	Nil vaccinated 15 (1.8%) unvaccinated	ICD-9 codes not described.
Conlin et al. (2013)[81]	Retrospective cohort	4122 (39.7%) H1N1 and 2745 (36.3%) trivalent	Monovalent H1N1 09 (10376)	Trivalent (7560)	2.1% of all trimester (H1N1) 2009 vaccinations. 2.0% of all trimester trivalent seasonal vaccinations	Case definition from the National Birth Defects Prevention Network.[62] ICD-9 codes for congenital anomalies (740.xx–760.xx).
Heikkinen et al. (2012)[87]	Mixed prospective retrospective	94 (4%)	Monovalent H1N1 09 (2295)	No vaccine (2213)	All trimesters adjusted OR 1.33 (95% CI: 0.88 to 2.00), adjusted for parity, smoking, and maternal age. 1st trimester 2 (2.1%) 2nd trimester 35 (2.7%) 3rd trimester 19 (2.1%).	All congenital malformations defined as such in EUROCAT guidelines.[63]

Study	Design	1st trimester vaccinations	Vaccination group (n)	Control group (n)	Results	Definitions
Horiya et al. (2011)[88]	Prospective cohort	15 (11.7%)	Monovalent H1N1 09, 2 doses (128)	Monovalent H1N1 09, 1 dose (82)	Nil vaccinated in 1st trimester. 5 (4.2%) vaccinated in 2nd and 3rd trimester.	No definition described.
Kallen and Olausson. (2012)[91]	Retrospective cohort	3165 (17.0%), weeks 1-9 1729.	Monovalent H1N1 (3165) Weeks 1-9 (1729)	No vaccine (138 931)	1st trimester only adjusted OR 1.01 (95% CI: 0.83 to 1.23). Adjusted for year of birth, maternal age, parity, smoking, BMI, preterm birth, low birth weight, and SGA <2 SD for age. Vaccination between weeks 1 and 9, OR 0.93 (95% CI: 0.81 to 1.22).	Any malformation registered in the Swedish Medical Birth Register
Mackenzie et al. (2012)[94]	Prospective cohort	Unclear	Monovalent H1N1 09 (104)	No vaccine (13)	6 in the vaccinated cohort.	No definition described.
Omon et al. (2011)[97]	Prospective single arm	29 (4%) < 15 weeks	Monovalent H1N1 09 (580)	N/A	All trimester vaccination. 19 (3.1%) major congenital malformations 7 (1.4%) minor cases or deformities	EUROCAT definitions.[63]
Opperman et al. (2012)[77]	Prospective cohort	55 (17%)	Monovalent H1N1 09 (Unclear)	No vaccine (1329)	All trimester, 'all malformations' adjusted OR 0.92 (95% CI: 0.58 to 1.46).† 1st trimester vaccination 'all malformations' crude OR 0.99 (95% CI: 0.43 to 2.00)	All malformations as per EUROCAT definitions.[63]
Pasternak et al. (2012)[46]	Retrospective cohort	345	Monovalent H1N1 09 (330)†	No vaccine (330)†	Adjusted POR 1.21 (95% CI: 0.60 to 2.45).‡	Major malformations as per EUROCAT definitions.[63]

Study	Design	1st trimester vaccinations	Vaccination group (n)	Control group (n)	Results	Definitions
Rubinstein et al. (2013)[83]	Cross sectional study	2874 (39.4%)	Monovalent H1N1 09 (7293)	No vaccine (23195)	All trimester vaccination. 35 (0.5%) vaccinated 137 (0.6%) unvaccinated	Alterations in anatomical development diagnosed during gestation or physical examination
Taveres et al. (2011)[103]	Prospective single arm study	42 (15.7%)	Monovalent H1N1 09 (267)	N/A	6 (1.9%) in all trimester vaccinations. Nil vaccinated in 1st trimester	CDC MACDP guidelines[64]
Deinard, Ogburn (1981)[78]	Prospective cohort	41 (23%)	Monovalent Hsw1N1 76 (176)	No vaccine (517)	14 (8.0%) vaccinated in all trimesters 67 (13.0%) unvaccinated cohort. ($p < 0.005$)	The data included major and minor malformations from nursery discharge summaries.
Sumaya, Gibbs (1979)[57]	Prospective cohort	Nil	Monovalent Hsw1N1 76 (56)	No vaccine (56)	3 had detectable congenital defects; inguinal hernia, phalangeal tag, and clubfeet.	No definition described.

† Propensity matched cohort sizes, refer to Appendix V.
‡ Propensity score adjusted, refer to Appendix V.

3.4.6 Small for gestational age (SGA) birth

Small for gestational age infants were reported in 8 studies.[46, 57, 80, 85, 91, 96, 101, 102] The studies included three investigating the trivalent seasonal vaccine,[96, 101, 102] four investigating the monovalent influenza A (H1N1) 2009[46, 80, 85, 91] vaccine, and one the (Hsw1N1) 1976 vaccine.[57] The definitions used for SGA birth consisted of SGA less than third percentile and SGA less than tenth percentile, or two or more standard deviations below the mean gestational age. Retrospective studies were included in meta-analysis as described in the 'assessment of heterogeneity' section. There were two retrospective studies combined in meta-analysis (figure 4) and they have been described previously.[46, 85] The influenza A (H1N1) 2009 analysis showed the Tau^2 was less than one, and combined with the I^2 percentage of 30% it indicates the studies are statistically homogenous. The meta-analysis indicated a statistically significant reduction in SGA less than tenth percentile births, although the upper confidence interval was close to the null value. Kallen and Olausson (2012) were not able to be included in meta-analysis as they used a different outcome measure of less than two SD. They had a point estimate favouring the unvaccinated cohort, with non-statistically significant confidence intervals.[91]

In the remaining retrospective studies one was investigating the influenza (H1N1) 2009 vaccine,[80] with the study by Richards et al. (2013) using SGA less than tenth percentile and reporting a point estimate that favoured the unvaccinated control group, although it was not statistically significant.[80]

SGA following vaccination with the seasonal vaccine was included in three other studies, with the secondary analysis of the randomised control trial by Steinhoff et al. (2012) indicating a reduction in the point estimate, with a non-statistically significant confidence interval.[102] The study by Sheffield et al. also on the seasonal influenza vaccine investigated both SGA less than the third and tenth percentile and reported that both with similar percentages and not statistically significantly different.[101] The remaining study on the trivalent seasonal vaccine conducted analysis in different periods of influenza circulation. The estimate during the putative period showed a non-statistically significant reduction in the point estimate that became a significant reduction when measurement was restricted to the peak influenza period.[96] The only study that investigated the monovalent (Hsw1N1) vaccine reported the percentage of SGA births from the vaccinated arm of the study only.[57]

The outcome of SGA infants had no statistically significant increase following vaccination. The larger population cohorts indicate that there is unlikely to be any adverse effect of gestational size of infants at birth associated with influenza vaccination.[85, 91, 98] Some studies and meta-analysis indicated that vaccination may reduce the number of SGA infants, especially during times of peak influenza circulation.[85, 96, 102] These findings would need to be confirmed in larger prospective research.

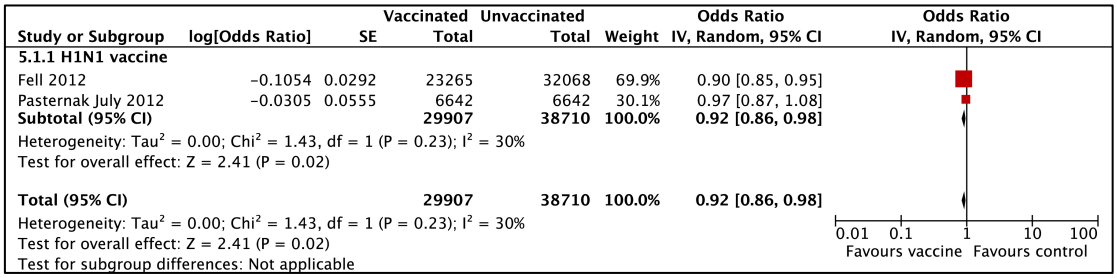

Figure 4 Meta-analysis of SGA < 10th percentile infant. Vaccinated versus unvaccinated pregnant women

Table 13 SGA infants following exposure of pregnant women to the influenza vaccine (not included in meta-analysis)

Study	Design	Vaccinated group (n)	Control group (n)	Result
Omer, et al. (2011)[96]	Retrospective cohort study	Trivalent (578)	No vaccine (3590)	Putative influenza season, OR 0.74 (95% CI: 0.47 to 1.15) Period of widespread influenza activity, OR 0.31 (95% CI: 0.13 to 0.75) Adjusted for gestational age at first antenatal visit, maternal diabetes, multivitamin use in pregnancy, history of alcohol use during pregnancy, education less than 12th grade, and mother married.
Sheffield et al. (2012)[101]	Retrospective cohort	Trivalent (8864)	No vaccine (76919)	SGA < 10th percentile, 944 (11%) vaccinated, 183 (11%) unvaccinated, (p=0.9) SGA < 3rd percentile, 311 (4%) vaccinated, 2579 (3%) unvaccinated (p=0.5)
Steinhoff et al. (2012)[118]	Randomised controlled trial	Trivalent (169)	Pneumococcal vaccine (167)	SGA <10th percentile, adjusted OR 0.63, (95% CI: 0.4 to 1.0). During the period with circulating virus, adjusted OR 0.43 (95% CI: 0.20 to 0.94) Adjusted for interval between vaccination and birth.
Fell et al. (2012)[85]	Retrospective cohort	Monovalent H1N1 09 (21363) + trivalent (1977)	No vaccine (32230)	SGA < 3rd percentile, Adjusted RR 0.81 (95% CI: 0.72 to 0.92) Adjusted for maternal age, family income, education, neighbourhood immigrant concentration, chronic hypertension, and maternal smoking.
Kallen, Olausson, (2012)[46]	Retrospective cohort	Monovalent H1N1 (18844)	No vaccine (84484)	< 2 SD, adjusted OR 1.08 (95% CI: 0.95 to 1.23) Adjusted for year of birth, maternal age, parity, smoking, BMI, preterm birth, and low birth weight.
Richards et al. (2013)[80]	Retrospective cohort	Monovalent H1N1 09 + trivalent (1064)	No vaccine (1505)	SGA < 10th percentile, adjusted OR 1.26 (95% CI: 0.94 to 1.69) Adjusted for: maternal age, asthma, gestational diabetes, cardiovascular disease, hypertension during pregnancy, multiple birth, pregnancy complication, any antiviral use during pregnancy, and site.

Study	Design	Vaccinated group (n)	Control group (n)	Result
Sumaya, Gibbs, (1979)[57]	Prospective cohort	Monovalent Hsw1N1 76 (56)	No vaccine (56)	SGA <2 SD, 9 infants (16%)

3.4.7 Low birth weight baby

Low birth weight infants were reported in 9 studies.[18, 46, 80, 83, 87, 91, 93, 102, 103] Only one study on the trivalent influenza vaccine included this outcome.[102] Low birth weight was consistently defined as less than 2500g in all of the studies. Only two of the studies assessed as suitable for meta-analysis had the outcome of low birth weight infant.[46, 91] For this outcome they were statistically heterogeneous with an I^2 statistic of 72% and the meta-analysis has not been included. This is the only outcome in which there has been an issue with the heterogeneity of the studies that have been combined. There are differences that may have resulted in the statistical heterogeneity for low birth weight. Pasternak et al.[46] has small numbers of pregnant women vaccinated during their first trimester and the analysis includes 2nd and 3rd trimester vaccinations only. Kallen et al[91] has a comparatively large number of first trimester vaccinations in their analysis. Potential confounders also have some difference with Pasternak et al, including several more variables in their propensity matched cohorts that were not assessed by Kallen et al. Some of these included history of foetal death, diabetes and other chronic conditions, drugs used during pregnancy, degree of urbanisation, history of birth defects, and spontaneous abortion. [46, 91] Both studies also used different methods for adjusting for potential confounders. Pasternak et al. adjusted odds ratio had a point estimate that favoured the unvaccinated cohort for pregnant women vaccinated during their second and third trimester, with a precise confidence interval that has upper and lower limits close to the null value.[46] Their separate analysis of pregnant women vaccinated during their first trimester had a point estimate that favoured the vaccinated cohort with wider non-statistically significant intervals.[46] Kallen et al. had a point estimate favouring the vaccinated cohort that was also not statistically significant.[91]

Remaining studies on the influenza A (H1N1) 2009 vaccine that had an unvaccinated comparator and calculated an odds or hazard ratio had a point estimate favouring the vaccinated cohort and confidence intervals that were precise, indicating no adverse effects of the vaccine on low birth weight were likely.[18, 83, 87 80] One of those studies indicated a statistically significant reduction in low birth weight infants in the vaccinated cohort.[83] One study that reported percentages for both cohorts had a reduced number of vaccinated women who gave birth to low birth weight infants.[93] The only study on trivalent seasonal influenza vaccine was from a randomised controlled trial, and the point estimate favoured the vaccinated cohort, but due to the low power had wide imprecise confidence intervals.[102]

The outcome of low birth weight infants had no statistically significant increased risk following vaccination and any adverse effect of the vaccine on low birth weight infants is unlikely. Results are described further in table 14.

Table 14 Low birth weight baby (<2500g) following influenza vaccination during pregnancy.

Study	Design	Vaccinated cohort (n)	Control group (n)	Results
Steinhoff et al. (2012)[102]	Randomised controlled trial	Trivalent (169)	Pneumococcal vaccine (167)	Adjusted OR 0.53 (95% CI: 0.2 to 1.4). Adjusted for gestational age at immunisation and interval from immunisation to delivery.
Hårberg et al. (2013)[18]	Retrospective cohort	Monovalent H1N1 09 (25976)	No vaccine (87335)	HR 0.90 (95% CI: 0.76 to 1.08). Adjusted for influenza exposure and vaccination status, age, parity, marital status, use of nutritional supplements during pregnancy, smoking during pregnancy, history of earlier foetal death, and eight chronic medical conditions.
Heikkinen et al. (2012)[87]	Mixed prospective retrospective	Monovalent H1N1 09 (2295)	No vaccine (2213)	Adjusted OR 0.88 (95% CI: 0.61 to 1.26). Adjusted for parity, smoking, and maternal age.
Kallen, Olausson, (2012)[91]	Retrospective cohort	Monovalent H1N1 (18 844)	No vaccine (84 484)	Vaccinated versus pre-vaccination group OR 0.91 (95% CI: 0.81 to 1.03). Adjusted for year of birth, maternal age, parity, smoking, BMI, preterm birth, low birth weight, and SGA <2 SD for age.
Lin et al. (2012)[93]	Retrospective cohort	Monovalent H1N1 (202)	No vaccine (206)	16 (7.9%) infants in the vaccinated group 28 (13.6%) in the unvaccinated group
Pasternak, et al. (2012)[46]	Retrospective cohort study	Monovalent H1N1 1st trimester (330) 2nd and 3rd trimester (6642)	No vaccine 1st trimester (330) 2nd and 3rd trimester (6642)	POR 0.83 (95% CI: 0.41 to 1.67), 1st trimester vaccination.† POR 1.14 (95% CI: 0.94 to 1.38), 2nd and 3rd trimester vaccination.†

Study	Design	Vaccinated cohort (n)	Control group (n)	Results
Richards et al. (2013)[80]	Retrospective cohort	Monovalent H1N1 09 + trivalent (1125)	No vaccine (1581)	Adjusted OR, 0.79 (95% CI: 0.56 to 1.10). Adjusted for: maternal age, asthma, gestational diabetes, cardiovascular disease, hypertension during pregnancy, multiple birth, any pregnancy or birth complication, and site.
Rubinstein et al. (2013)[83]	Cross sectional	Monovalent H1N1 09 (7293)	No vaccine (23195)	Adjusted OR 0.74 (95% CI: 0.65 to 0.83). Adjusted for: antenatal visits, level of education, maternal age, income, parity, smoking, and history of pregnancy induced hypertension.
Taveres et al (2011)[103]	Prospective single arm	Monovalent H1N1 09 (267)	N/A	21 (8.1%), 12 were preterm.

† Propensity matched cohorts, refer to Appendix V.

Chapter 4. Discussion and Conclusion

Introduction

Chapter 4 will further explore and discuss the results of the systematic review and draw conclusions from the findings. The premise of this review is that pregnant women are a unique group due to the changes that take place to their immune system during pregnancy, as well as the potential effects of the influenza vaccine on the growing foetus and infant. Although some evidence gathered from research on healthy adults is applicable, the effectiveness and safety of vaccines in pregnant women should be considered separately, just as it is in other groups with altered immune function such as children and the elderly.

It is clear from this systematic review that there has been a large body of evidence added in the three years following the influenza A (H1N1) 2009 pandemic. It is difficult to assess how relevant the evidence gathered during this period is to the trivalent seasonal vaccine, and if it is transferrable to our next pandemic vaccine and outbreak. The increased severity of illness in pregnant women, mixed with the good match between the vaccine and circulating pandemic strain, should have resulted in increased protective effect if there is one. Any association with a protective or adverse effect during the pandemic outbreak may not be replicable during a non-pandemic influenza season, or with a polyvalent vaccine.

4.1 Effectiveness in pregnant women

Recent studies on pregnant women and the influenza vaccine have shifted focus to the effects on the foetus and infant, rather than the mother. Only a small number of studies are available that demonstrate the vaccines are effective at preventing influenza in pregnant women, and an even smaller number assessing the effects on reducing severe illness and mortality in pregnant women. This remains an important area, with complications from influenza during the influenza A (H1N1) 2009 pandemic resulting in increased mortality and severe illness in pregnant women.[9, 13]

There is only one recent randomised controlled trial that is able to give us an estimate of the seasonal vaccine's effectiveness in reducing influenza-like illness in pregnant women. A systematic review on the effectiveness of trivalent influenza vaccination on healthy adults by Jefferson et al. (2010) reported an estimated effectiveness at 30% (95% CI: 17% to 41%) during times that the vaccines were well matched.[32] That estimate is similar to the effectiveness estimated in the sole randomised controlled trial in this review investigating this outcome.[59]

Estimating effectiveness of outcomes generally requires the cohorts to be at similar risk of harm. There were four retrospective studies that reported results on the effectiveness of the vaccine in reducing influenza-like illness in pregnant women.[7, 18, 80, 95] Risk of contracting influenza fluctuates with the peaks

and troughs of virus circulation during an influenza season and varies with the lengths of time exposed to the virus. The studies need to take this into account with their design and one method is to use a model that involves survival analysis,[119] as performed by Hårberg et al. (2013)[18] Another method is to match the unvaccinated cohort at a similar stage of pregnancy, length of time exposed to the influenza season, and timing, in relation to the peak period of circulating virus. This needs to be considered when looking at outcomes from individual retrospective studies. Even with consideration of these factors there are still important limitations with retrospective designed studies that require passive identification of cases, and in particular non-hospitalised cases.[105] Ideally prospective research that has primary end points which include laboratory confirmed cases of influenza, rather than influenza-like illness, should be conducted to establish effectiveness of the vaccine.[105]

Research into the effectiveness of the influenza vaccine was sparse. Conducting prospective research during a pandemic is extremely difficult due to the limited time to design the study, as well as arrange funding and ethics approval. In addition, the methods used for testing are inconsistent as resources, both human and material, become scarce so that sets of symptoms are used to diagnose positive cases and laboratory viral testing is restricted to prioritised groups, or ceases all together. Despite these obstacles, large amounts of public money is spent developing and administering influenza vaccines. More prospective research is required to better inform decision makers and the general public. It is logical that reducing non-hospitalised influenza-like illness or influenza should result in a decrease of severe disease. However as yet there is currently no evidence available to show that this is the case. This is an important area of research not only for the health of pregnant women, but also for foetal outcomes as hospitalised maternal illness is currently considered the main threat to good outcomes.[18] Reducing severe disease in pregnant women appears to be one of the highest priority areas for future research.

4.2 Effectiveness for infants up to 6 months of age

Effectiveness of the vaccine in reducing both influenza symptoms and hospitalisation for infants up to 6 months of age appears to be important evidence as to why pregnant women should get vaccinated during non-pandemic influenza seasons. Studies conducted on the seasonal vaccine by Eick et al. (2011), Poehling et al. (2011), Zaman et al. (2008), and Benowitz et al. (2010) demonstrated a clinically significant protective effect for infants up to 6 months of age.[37, 59, 84, 99] Generalisability of findings in both the randomised control trial by Zaman et al. (2008) and the prospective cohort study by Eick et al. (2011) is an issue due to the location and demographics of the participants. As well as this, three of the studies were conducted on participants during their second or third trimester and it is not clear if it would be equally as effective for pregnant women vaccinated during their first trimester. Like all influenza vaccines, they are also likely to be more effective when they are well matched to the circulating

influenza strains, and less so when the match is poor.[32] Further studies in this area would be of benefit to enable clinicians and policy makers to quantify how effective they can be in different population groups, settings, seasons, and vaccine types. In Australia a large National Health and Medical Research Council funded study (FluMum) is conducting an observational study of 10106 mother and infant pairs that will add to the body of evidence for the effectiveness of reducing influenza in infants less than 6 months of age through maternal vaccination. As well as this study, clinical trials are also being conducted in South Africa, Nepal, and Mali.[120]

Hospitalisation of infants was investigated by two case-control studies that were conducted in similar population groups in the USA,[84, 99] and whilst they are considered lower level evidence due to their retrospective case-control design, they provide a compelling argument for expecting mothers to strongly consider vaccination. It is not possible to accurately calculate the numbers needed to vaccinate to prevent a hospitalisation of an infant. However, even a small reduction in severe illness and hospitalisation amongst newly born infants up to 6 months of age would be of significant clinical benefit.

4.3 Adverse events for pregnant women

Both clinicians and pregnant women need to have a clear understanding of the potential adverse events that may be encountered following influenza vaccination. This outcome was specifically targeted at events affecting the pregnant women, and not the foetus. Explanation of the potential adverse events and appropriate management of symptoms, especially fever in the first trimester, should be an important part of the vaccination procedure.

There are systems in place in many countries that conduct passive surveillance on adverse event reporting, including pregnant women. These systems provide an indicator for clusters of events, trends or severe adverse events, but do not replace the need to conduct targeted research on the outcome as these systems are prone to under reporting.[121] However, due to the large sample size needed to detect rare conditions such as Guillain-Barré Syndrome, this review is unable to detect small variations in the incidence of such conditions. A systematic review conducted by Jefferson et al. (2010) on influenza vaccination in a healthy adult population cited evidence that estimates the incidence of vaccine related Guillain-Barré Syndrome at 1.6 extra cases per million vaccinations.[32] The numbers of pregnant women included in the adverse event outcome were not large enough to assess changes this small, however, there were no cases reported.

There were a limited number of studies investigating adverse events for pregnant women with the seasonal vaccine. Symptoms such as fever and fatigue are important factors to consider for clinicians caring for pregnant women and the women themselves. Accurate information should be available so that women are well informed about common local and systemic reactions.

Studies investigating adverse events with the influenza A (H1N1) vaccine and preeclampsia did not indicate a statistically significant association with vaccination. However, it is biologically plausible that the inflammatory response from influenza vaccination during pregnancy could be associated with developing preeclampsia.[122] A study by Christian et al. (2012) demonstrated statistically significant increases in C-reactive protein (CRP) on the first and second day following vaccination, indicating a substantial inflammatory response. Inflammatory processes may contribute to gestational hypertension and preeclampsia.[122] Whilst there is no evidence that there is an increased association, these findings do not rule out an association and further research on this outcome is required.

4.4 Foetal, birth and infant outcomes

Birth outcomes have been the area of largest growth in evidence over the last three years. The study designs included in this review were mostly retrospective studies geared towards investigating the safety of the vaccine on birthing outcomes. Retrospective studies investigating foetal, birth, and infant outcomes are complex due to the multi-factorial risks and causes of spontaneous abortion, premature birth, congenital abnormalities, SGA infants, and low birth weight infants. Each outcome has multiple potential confounding factors that may impact on these outcomes. Confounding is a threat to the validity of studies, and in particular observational studies.[123] Confounding can result in the over or under estimation of outcomes and is potentially an issue with many of the studies in this review. The risk is magnified with the use of 'all cause' outcomes that don't decipher if the women with the outcome of interest were exposed to influenza. There was varying quality of the studies included in regards to the matching of cohorts and/or analysis and adjustment of potential confounding factors. This was a significant issue with many missing important variables that may increase the risk of poor birthing outcomes.

Influenza vaccination during pregnancy did not have an association with or increase the incidence of premature birth. It was one of the outcomes with the most evidence available and also had a consistent definition that made it easier to compare studies. It is possible that influenza vaccination during pregnancy provides some protection during an influenza season, although there are many caveats as previously discussed.

Foetal death following influenza vaccination during pregnancy had a variety of definitions that complicated comparison between studies. Large cohort studies indicate that there is unlikely to be an association between the influenza vaccine and adverse outcomes due to foetal death.[18, 83, 85, 91, 98] All but one of the large cohort studies was investigating the seasonal influenza vaccine.

There was also sufficient evidence to conclude that influenza vaccination during pregnancy was unlikely to have an adverse effect on gestational growth and birth weight.

Spontaneous birth and congenital malformations were two outcomes that required accurate information on the timing of influenza vaccination and date of conception, as the first trimester is a crucial stage of foetal development and key to these outcomes.[124] Precise measurements for these outcomes were affected due to reduced statistical power with less women getting vaccinated during their first trimester. Some studies reported outcomes for congenital malformations in women vaccinated during any trimester, and whilst this is applicable for women vaccinated during their second and third trimester, it does not equate to evidence that it is safe in the first trimester when the foetus is at greatest risk from teratogenic agents and effects, including fever. Both of these outcomes are of important clinical significance considering even a minimal increase in either outcome would likely outweigh the benefit of getting vaccinated during the first trimester of pregnancy.[5] Clinically there is no evidence that the vaccine is unsafe to use in the first trimester. However the evidence for spontaneous abortion and congenital abnormalities would be strengthened with increased sample size of the vaccinated cohort and comprehensive assessment.

4.5 Trimester of vaccination

The risk of harm to pregnant women from contracting influenza increases as the pregnancy progresses to the second and especially the third trimester. The risk to pregnant women from influenza during their first trimester is only marginally more than the healthy non-pregnant population.[5] Recommending the influenza vaccine is based on an assessment of the risks and benefits, and multiple factors should be considered when assessing the evidence for which trimester is the most effective and safest to be vaccinated. The only evidence for reducing rates of influenza in infants less than 6 months of age is with pregnant women vaccinated during their second or third trimester. When this evidence is combined with the increased statistical power and clinical certainty of the safety of the vaccine being administered during the second or third trimester, it is appropriate that vaccination during the second and third trimester be the recommended clinical practice. However, there are occasions when vaccination during the first trimester will need to be considered. This occurs when there is increased harm from the virus, such as a pandemic outbreak, or the pregnant woman has comorbidities that place her and her foetus at greater risk, or the timing and severity of the peak influenza period is a factor.

These findings do not necessitate a change to current recommendations for the vaccine. However, it seems prudent to provide a prescriptive statement on the most effective and safest trimester to be vaccinated, in line with best available evidence. This may provide clinicians and pregnant women with clarity and confidence regarding the optimal timing of influenza vaccination in maximising effectiveness and safety.

4.6 Findings from the wider review literature

Only one systematic review was available for comparison on the effectiveness of the seasonal influenza vaccination during pregnancy.[125] The authors conducted a search of the Cochrane database of systematic reviews, Cochrane Central Register of Controlled Trials, and Medline from 2006 to 2011. From their search they found no systematic reviews on the effectiveness or risks of influenza vaccination during pregnancy. One randomised controlled trial by Zaman et al was included and it was considered it to be of high methodological quality.[125] The authors used the GRADE classification method and considered it level B evidence for effectiveness against respiratory tract infections and fever. They also reported that the studies were from subtropical and less developed countries and *"high-quality studies to confirm these effects in temperate regions are urgently needed. Pregnant women and their newborns might benefit from influenza vaccination, but large studies in temperate climate zones are still needed."*(p.9169)[125]

Findings from that sole systematic review are restricted to the sole randomised controlled trial that is also included in this review, thus the findings are not dissimilar to the ones made in this review. A search of Pubmed and Google Scholar did not locate any other systematic reviews post 2011.

4.7 Study quality

Study quality and the type of studies that made up the evidence in this review impacts on the conclusions that can be drawn. Even though critical appraisals were performed and only studies that contained five or more criteria were retained in this review, there remains high variability in the level of quality of the studies retained. Raising the number of criteria required for the study to be retained in this review may have rectified this, but it was thought that being more inclusive of the studies on influenza vaccination during pregnancy would provide more informative and transparent evidence.

4.7.1 Size and duration

The duration of studies varied, as you would expect with so many studies included with different aims. Because influenza is seasonal in temperate regions, it was quite common for studies to span one influenza season, especially those investigating outcomes for the influenza A (H1N1) 2009 influenza vaccine. Studies that were investigating adverse events for pregnant women were also mostly conducted over one influenza season with one specific type of vaccine. Some of the studies that investigated the effectiveness and safety of the seasonal vaccine spanned three or more influenza seasons, [7, 37, 79, 84, 95, 99, 101] and potentially have greater generalisability than those performed over just one season. However, ideally they should indicate how well the vaccine matched the circulating strain and the finding would be better able to be extrapolated and compared to other results.

Follow up time was dependent on the aim of the study and no issues were found for this, with reasonably clear cut timelines for outcomes i.e., follow up until birth. In this review, studies were only included if they contained outcomes for children under the age of 6 months. There were some studies that were excluded, as they did not contain separate data for that time frame.[126] Ideally studies should use 6 months of age as at least a sub-group, as children can start to be vaccinated at 6 months of age.

Sample size was an issue for some studies, especially when investigating uncommon events in regards to safety. This was particularly evident with investigations on the effects of influenza vaccination during the first trimester. This was due to difficulty ascertaining dates that vaccination occurred, or just generally low rates of vaccination in this group affecting the precision of some of the results, including some of the higher quality retrospective studies. This makes interpretation difficult as the upper confidence intervals can indicate that there is a possibility of harm.[127] Some studies included an entire pregnant population overall enabling good statistical power for most of the less common outcomes, especially during the second and third trimester of pregnancy.

4.7.2 Conflict of interest of study authors

There are 16 studies included in this review for which one or more of the authors declared they had received pharmaceutical industry support or payment. Potential conflicts of interest were unclear in seven studies, with most being from the older published material, and the remaining 16 reported no conflict of interest. Potential conflict of interest has been found to be a concern with previous influenza vaccination systematic reviews.[32] Jefferson et al. reported an inverse relationship between risk of bias and direction of study conclusions, and favourable conclusions were associated with a higher risk of bias.[32] The authors also found that industry funded studies were more likely to draw favourable conclusions and be published in higher impact journals than those not funded by industry.[32]

4.7.3 Study populations

On the whole, the sampling of participants in the cohort studies was representative of the pregnant population during their second or third trimester of pregnancy, due to large population-based cohorts. Women were included with other factors that placed them or their offspring at risk from complications from influenza. Adverse event investigations for pregnant women contained several studies that were performed on healthy pregnant women only. Many of these were primarily immunogenicity studies with adverse events as a secondary outcome measure.

Two of the key studies investigating the effectiveness of the influenza vaccine were conducted on women from Bangladesh and an Apache Indian Reserve in the USA. Both populations groups are potentially from socioeconomic disadvantaged groups. The studies on these populations groups may not be entirely generalizable to urbanised populations in developed countries where the vaccine is

predominantly used. As previously mentioned, pregnant women vaccinated during their first trimester were under-represented in the study cohorts. This population group requires a concerted focus as there are logistical difficulties studying women vaccinated during the first trimester of pregnancy and it is one of the main areas where further evidence is required.

4.7.4 Measurements

Foetal death, congenital malformation, and spontaneous abortion had an array of different definitions. Definitions for foetal loss included:

- groups of ICD-9 codes,
- foetal death in women > 20 weeks gestation,
- miscarriage or stillbirth after 12 completed weeks of pregnancy,
- foetal death after 22 weeks gestation,
- foetal death with weight 500g or more, and
- five with no definition.

Definitions for spontaneous abortion included:

- foetal death prior to 20 weeks,
- foetal death prior to 22 weeks gestation,
- spontaneous pregnancy loss occurring at, or before, 16 weeks of gestational age,
- foetal death between 7 and 12 weeks, and
- foetal death prior to 24 weeks.

Consistent definitions would aid in the synthesis of study results and the variety of definitions used means comparing outcomes is problematic and meta-analysis is not possible.

4.7.5 Potential confounders

Confounding is a threat to the validity of studies, and in particular observational studies.[123] Confounding can result in the over or under estimation of outcomes and is potentially an issue with many of the studies in this review. The risk is magnified with the use of 'all cause' outcomes that don't decipher if the women with the outcome of interest were exposed to influenza. There was varying quality amongst the studies included in regards to the matching of cohorts and/or analysis of potential confounding factors. This was a significant issue with many of the large retrospective cohorts, with many missing important variables that may increase the risk of poor birthing outcomes. This is in part due to the use of medical databases. There is a 'trade off' for having large sized cohorts and in most cases data is derived from computerised medical information that is already coded. The most common variables assessed in the large retrospective cohorts were those easily collected from the databases, such as maternal age and chronic diseases. The least common were information that would have probably involved investigation

of client medical records, or use of other methods to obtain information such as socioeconomic status, smoking, alcohol and drug use. Even if every variable above was included in analysis, birthing outcomes such as premature birth are areas in health in which much remains unknown.[106] Causality and purported risk factors have been difficult to prove, with some risks requiring cofactors to exert their effect and many preterm births occur without any risk factors.[106]

4.8 Issues regarding observational influenza vaccine research

There is increased interest and need in pharmacological research to monitor health outcomes in real world settings, rather than just rely on studies investigating the efficacy of a drug.[115] The JBI approach to systematic reviews of effectiveness is congruent to this approach, by having an inclusive approach to the type of studies that provide evidence for outcomes of this nature. Experimental studies that aim to establish a causal relationship between two variables, and observational studies that imply a correlation or a relationship between two variables are used depending on the nature of the evidence sought.[73]

Similar to research on the influenza vaccine in the elderly, the bulk of the evidence for the influenza vaccine for both pregnant women and infants under the age of six months is derived from observational rather than experimental evidence. Whilst there are some positive aspects of sourcing evidence from observational studies including better generalisability, including inclusion of people with comorbid diseases and women vaccinated during their first trimester, there are limitations. Systematic and serious overestimation of the effectiveness of the influenza vaccine in the elderly highlighted potential issues of confounding with influenza research, especially observational studies.[105] Much of the evidence that indicated that the influenza vaccine saved approximately one life for every 200 vaccinations was found to be unreliable and the real protective effect was far less than initially thought.[105] This was thought to be due to selection bias with the presence of a sub-group of seniors who were not vaccinated due to being frail or terminally ill.[105] Using ‘all-cause’ outcomes such as ‘all-cause mortality’ in winter magnified this bias. Even though ‘all-cause mortality’ is not likely to be an outcome investigated during seasonal influenza seasons for pregnant women, all cause outcomes for birth and infant outcomes are commonplace in this review.

Observational research rather than experimental is likely to remain the mainstay of evidence available for women vaccinated during pregnancy, due to the comparative cost and ethical considerations as the vaccine is already recommended in most countries. This is no different to studies for the influenza vaccine in other risk groups such as the elderly.[105] Lessons should be learnt from influenza research in other risk groups and some of the studies included in this review did follow some of the recommendations made for influenza research on the elderly. Key recommendations from Simonsen et al. are made for vaccine effectiveness and cost-effectiveness research in the elderly. They include:[105]

- Abandoning convenient electronic cohort studies in favour of primary observational studies with laboratory confirmed end points, as well as comprehensive assessment and adjusted for potential confounding factors by manual chart review.
- Carefully controlled inpatient studies of laboratory confirmed influenza and influenza vaccination rates.
- Manual chart review with a specific end point i.e. x-ray confirmed pneumonia hospitalisations, and controlled for bias by adjusting the relative risk to 1 during the pre-influenza period.

The unique aspect of researching influenza is that in most temperate climates there is an influenza period that allows the measurement of an influenza related event in a period when there is no virus circulating. Risk of contracting influenza fluctuates with the peaks and troughs of virus circulation during an influenza season and varies with the length of time exposed to the virus. The retrospective studies need to take this into account with their design. One method, especially for studies looking at outcomes during the first trimester, is to use a model that involves survival analysis.[119] Another method is to match the unvaccinated cohort at a similar stage of pregnancy, length of time exposed to the influenza season, and timing in relation to the peak period of circulating virus.

Due to the age of pregnant women they are less likely to have as many serious comorbidities and medication use that may confound all cause outcomes. However, there are numerous variables that may increase the risk of poor birthing outcomes. All cause outcomes of premature birth, small for gestational age, congenital abnormalities, foetal death, spontaneous abortion, and low birth weight were all recorded in studies contained in this review, without any indication that the pregnant women had influenza at any stage during their pregnancy. Even with regression analysis and adjustment for some of the factors mentioned there still remains concerns about the quality of the information.

For influenza vaccination in the elderly it is thought that *"simply applying more sophisticated confounder modelling techniques that use the existing database variables alone will probably not suffice and cannot substitute for accurately measuring important confounders. The use of propensity scores, for example, does not reduce bias due to unmeasured confounders and generally produces similar results to standard regression adjustment that involves the same database variables".* (p.691)[109]

Some of the retrospective studies included in this review followed recommendations suggested for influenza research in older people, although they were in the minority. Two case-control studies conducted controlled inpatient studies of hospitalised children with laboratory confirmed influenza.[84, 99] One study adjusted for potential bias by using models that were based on the approach of identifying covariates that produce adjusted ORs of 1 during the pre-influenza period at a time where there should be no effect.[96] One study investigated whether the associations were due to underlying differences

between individuals who were vaccinated and those who were not (a ‘‘healthy user effect’’).[100] They compared a period when influenza was not circulating widely and little or no protective association with foetal death should be observed. Any association that was observed was considered to be an estimate of the level of confounding present in their modelled estimates. They found that the protective association with influenza A (H1N1) 2009 vaccine with foetal death was similar during periods of high influenza circulation and during periods of little or no influenza circulation.[100] They investigated and analysed several potential confounding factors including maternal age, history of spontaneous loss, diabetes, pre-pregnancy smoking status, pre-pregnancy alcohol use, pre-pregnancy body mass index, the number of consultations in the 6 months before the last menstrual period date, and being in an influenza A (H1N1) clinical risk group (i.e. chronic condition). The authors concluded that it was suggestive of unmeasured confounding as the vaccine should not be providing any true protective effect in these periods, and that, *“developing methods to account for, or evaluate, residual confounding will be vital in any [future] such studies.”* (p.e51734)[100]

4.9 Systematic Review Methods

This systematic review had some strengths and limitations. A protocol defining the inclusion and exclusion criteria (population, intervention, comparator, and outcomes) was used as described in the systematic review protocol. A comprehensive search was conducted including grey literature as described in Appendix I, and this potentially reduces the risk of publication bias. Two reviewers independently using predefined and validated tools critically appraised the studies. As a result 39 studies were included across both outcome measures for effectiveness and safety.

At the time of developing the protocol it was decided to include information about adverse events and birthing outcomes, as well as outcomes for effectiveness. This decision was made so as to provide a more complete set of evidence for people to use when making policy, prescribing, administering, and having the influenza vaccine during pregnancy. Authors of systematic reviews have previously focused on beneficial outcomes of interventions, and systematic reviews on harms have been less common.[127] Both benefits and harm are important pieces of evidence and necessary to make any decision when weighing up the balance between both. This proved to be important with much of the new evidence obtained during the influenza A (H1N1) pandemic coming from studies investigating birthing outcomes.

This review included both experimental and epidemiological study designs including randomised controlled trials, non-randomised controlled trials, quasi-experimental, prospective and retrospective cohort studies, case control studies, and analytical cross sectional studies. That resulted in the collation of a comprehensive and inclusive body of evidence. This is potentially a strength and a limitation. The studies used in this review are the same studies that currently inform policy decisions, clinical decision,

and personal decisions in regards to getting vaccinated during pregnancy. To restrict the studies included in this review to randomised controlled trials or prospective experimental studies would have severely restricted the amount of evidence available for this review and ignored research that is already informing policy. Critically appraising the evidence and synthesising it in a systematic review is an important missing part of evidence regarding influenza vaccination during pregnancy.

The broad array of study designs and the large number of observational retrospective studies does however result in limitations to what conclusions can be drawn and synthesising the evidence. As previously discussed, observational, or epidemiological population based study designs, are subject to biases and confounding.[123] Some researchers are critical of the worth of these types of studies for informing healthcare.[128] There is some basis to this argument, however this topic is a good example of the problems associated with just restricting evidence to randomised controlled trials. Influenza for both pregnant women and their infant can be a serious disease and currently influenza vaccination is the primary preventive measure to protect mothers and infants. [27] Currently around the world influenza vaccination during pregnancy is recommended in most countries. This recommendation is not based on one available well-conducted randomised control trial by Zaman et al.[59] In the USA they were vaccinating pregnant women close to a decade prior to this piece of research.[29] Even though the RCT provides excellent information to guide decision-making, there are multiple sources of evidence used to make decisions regarding the influenza vaccination of women during pregnancy. The vast majority are not randomised controlled trials. There are times when observational studies provide important evidence that a randomised controlled trial may not be able to. The safety of influenza vaccination during pregnancy is a good example, where large numbers are needed to investigate rare outcomes. For many of these outcomes such as foetal death and congenital malformation, randomised controlled trials are often designed to investigate a hypothesis evaluating the effectiveness of an intervention and may lead to inaccurate conclusions about adverse events.[129] Even though randomised controlled trials are the gold standard for investigating the effectiveness of an intervention, they are often smaller sample sizes due to logistical and financial constraints, and these may result in reduced statistical power to be of use in informing outcomes for adverse events. Even though observational research contained in this review is important and does provide useful evidence, there is little doubt that more prospective and experimental research is required for many of the outcomes assessed in this review to further inform researchers, clinicians, policy makers, and the general public.

As well as its strengths, this review also has some limitations. Even though the review aimed to be as inclusive as possible, not all evidence was assessed and this means that not all evidence was used to provide information about influenza vaccination during pregnancy. Evidence derived purely from national or regional vaccination surveillance was excluded from this review for two main reasons. The

task of locating and collating studies and grey literature that contained surveillance information from around the world would have been a monumental task. Even though surveillance provides an indicator for clusters of events, changing trends, or severe adverse events, it is prone to under reporting.[121] From a systematic review perspective it was thought that the level of evidence quality was not suitable or practical for this review. Another missing source of evidence is case reports. Case reports can identify uncommon adverse drug events that often go undetected in clinical trials.[129] In the case of this review it was thought that study design was not going to make a large contribution to the review, considering the length of time the vaccine has been manufactured and used. However, with changes in manufacturing methods, changes in strains in the vaccines, and introduction of new adjuvants, it could be argued that case reports would have been of some value.

Evidence on the efficacy of the vaccine at producing an immunologic response in pregnant women and/or infants under the age of 6 months was not included in this review. Some immunologic studies were included in this review, although only data regarding adverse events was extracted. This evidence is potentially useful to compare the immunologic responses in pregnant women. Research has shown that transplacental transfer of antibodies to infants occurs,[55, 130, 131] and further information from these types of studies may better inform about adjuvants used, the timing of vaccination, and duration of potential protection. Evidence obtained from animal modelling was also not included, which can provide some evidence on potential teratogenic effects of medications and vaccines.

Heterogeneity between studies has meant that meta-analysis has not been able to be performed for all available studies and outcomes. This has resulted in the tabling and narrative summaries for many of the outcomes. Whilst every effort has been made to remain objective and transparent, the nature of combining narrative results and forming a conclusion is open to subjective assessment and potential bias from the authors.[132] Using JBI methodology and tools that included a systematic search, critical appraisal, and data extraction tools, every effort has been made to minimise this risk.

Meta-analysis has been performed on observational studies that were deemed suitably matched to be combined. Meta-analysis of observational studies carries a risk of overestimation or underestimation due to combining errors in measurement of the exposure variables, confounding, and biases, that do not usually occur in randomised controlled trials.[108]

A comprehensive search for studies was undertaken, including for grey literature. However, searching was only conducted in English, meaning that some important papers written in another language may be available, but not included in this review.

4.10 Implications for research

4.10.1 Research investigating the effectiveness of the vaccine for pregnant women

There are several implications for future research, with many building on the evidence derived from existing observational studies. Implications for further research involving the effectiveness of the influenza vaccine for pregnant women include:

- Use a prospective design with a large enough sample size to ensure that there is adequate statistical power to be able to calculate accurate numbers needed to vaccinate.
- Good quality prospective studies need to be performed in developed countries to ascertain if the vaccines are as effective as the trials done in developing countries, or in communities of lower socio-economic background.
- Prospective research that has primary end points, which include laboratory confirmed cases of influenza, rather than influenza-like illness.
- Case-control studies, or retrospective cohort studies, that involve manual chart review of hospitalised pregnant women and identification of laboratory confirmed influenza, or a specific end point i.e. x-ray confirmed pneumonia, rather than reliance on ICD coding.
- Adjust for important confounders in prospective unmatched cohort studies and retrospective studies. To be comprehensive this will more than likely need to involve direct interview or at the very least manual chart review of potential confounding factors.
- Report the match of the circulating influenza strain with the influenza vaccine used during the study period.
- Studies on effectiveness should not include adverse event outcomes if the design is not appropriate i.e., calculation of appropriate sample size to measure adverse events as an outcome was not considered at the planning stage of the study.
- The usefulness of retrospective observational studies to ascertain the effectiveness of the influenza vaccine in non-hospitalised pregnant women is limited. Unless an emergency situation occurs, such as was the case with the 2009 influenza A (H1N1) pandemic, further studies of this type should be avoided.
- If retrospective studies investigating the effectiveness of the vaccine are performed, then a time dependent hazard ratio should be used.
- If retrospective studies investigating the effectiveness of the vaccine are performed, then a concise influenza period should be used to avoid large variations in circulating virus.

- Even though it is logistically difficult, planning, ethics and funding should be set aside for the next pandemic, so that prospective research can be conducted during the pandemic period, especially investigating the protective effects of the vaccine.

There is currently no research that demonstrates pandemic or seasonal influenza vaccines reduce the severity of illness or hospitalisation of pregnant women. This area is a priority as hospitalised maternal illness is currently considered the main threat to good foetal outcomes, as well as detrimental outcomes for pregnant women.

4.10.2 Research investigating the effectiveness of the vaccine for infants under 6 months

Implications for research in children up to 6 months of age are similar to those listed for pregnant women. More research has been conducted in this group regarding the effectiveness of the vaccine at reducing hospitalisation, however further studies need to be conducted with a prospective design and ideally allow a precise absolute risk measurement, so that a number needed to vaccinate can be estimated. Prospective research on the effectiveness of the vaccine in this group should also consider ensuring that the trimester of vaccination is recorded, and that there is a large enough sample size for children born to mothers vaccinated during their first trimester to give a precise measurement. The sample size should also be large enough to ascertain what trimester of pregnancy provides the most effective reduction in influenza in children up to 6 months of age.

4.10.3 Research investigating adverse events in pregnant women

The number of studies included in this review that were investigating adverse events for the influenza A (H1N1) vaccine compared to seasonal influenza vaccine were in the ratio 4:1, which is probably due to the increased priority of protecting pregnant women during the pandemic. Although, considering that the pandemic vaccine was only manufactured in 2009 and the seasonal vaccine has been in production for decades and recommended for pregnant women in the USA for around a decade, it does appear disproportionate. The findings for the influenza A (H1N1) vaccine may not be generalisable to the trivalent vaccine due to it being a monovalent vaccine. Larger prospective studies with the trivalent seasonal vaccine need to be conducted to ascertain an accurate incidence of adverse events, especially considering pregnant women remain the top priority group for vaccination with the influenza vaccine. Future studies on the safety of influenza vaccine should not just be secondary outcomes of immunologic studies, they should also be designed for the purpose of identifying adverse events with enough numbers to be informative for less common outcomes. Further research is also required for the potential effects of the influenza vaccine, specifically on preeclampsia.

4.10.4 Research investigating birthing outcomes

The majority of research on birthing outcomes was conducted on the influenza A (H1N1) vaccine during the 2009 influenza pandemic. The majority are retrospective studies that are only suitable to imply an association and designed to investigate the safety of the vaccine. The findings in these studies regarding the safety of the vaccine may not be generalisable to the seasonal vaccine. There are retrospective cohort studies that imply there is a protective association for birth outcomes such as premature birth and foetal death. Large prospective studies are needed to confirm that vaccinating pregnant women reduces the risk for these outcomes.

Implications for further research involving birthing outcomes include:

- Further studies that investigate women vaccinated during their first trimester for spontaneous abortion and congenital abnormality outcomes with adequate sample sizes are needed.
- The use of standard definitions for foetal death, spontaneous abortion, and congenital abnormalities would help with comparing study results.
- Adjust for important confounders. To be comprehensive this will more than likely need to involve direct interview, or at the very least a manual chart review of potential confounding factors.
- Use of primary end points that are not 'all-cause' outcomes and include mothers with laboratory confirmed cases of influenza.
- Use of a large prospective designed study with enough statistical power to assess less common birthing outcomes.

4.10.5 Research investigating the effects of pregnant women contracting influenza

This systematic review did not conduct a systematic search of the literature or extract every finding available regarding the risks of contracting influenza during pregnancy. It does appear that further epidemiological research may be useful in this area to better inform health professionals and the public. In seasonal influenza seasons the risk to pregnant women appears to be less than that of the elderly and children,[3] although harm for both the mother and infant aged less than 6 months of age needs to be considered. The focus on pregnant women as the number one priority group may be detrimental to groups who are at more risk of harm, as well as cause unnecessary alarm. There is conflicting evidence on the harm caused from contracting influenza during pregnancy and potential harm to the foetus. Some retrospective observational studies are finding an association between the influenza vaccine and protective effects for some birthing outcomes. Further epidemiological research may be able to shed light on the extent, if any, influenza contributes to adverse birthing outcomes i.e., is exposure to influenza during pregnancy associated with or cause premature birth? There was some research that

suggested children whose mother was vaccinated during pregnancy had a reduced immune response to the influenza vaccine at 6 months of age, and this also needs further investigation.[36]

4.11 Implications for practice

Rates of vaccination for women during pregnancy during the 2009 influenza A (H1N1) pandemic ranged between 0% to 54% in Europe, with the majority of countries estimating rates between 0% and 25%.[18, 45, 46] This was during a period where women were thought to be at an increased risk of harm from complications of influenza during the 2009 pandemic. The results of this systematic review supports efforts to vaccinate pregnant women during their second and third trimester of pregnancy. Health professionals can be confident that the vaccine has been shown to be safe and effective for women during this period. However, influenza vaccination during the first trimester of pregnancy may be warranted during pandemic periods, or for women with comorbidities, or dictated by the timing and severity of the peak seasonal influenza period. This decision needs to be made in consultation with the pregnant woman, noting that women with comorbidities are at an increased risk of harm. [3, 5, 14, 15]

Health professionals should advocate for the safety of the vaccine when discussing vaccination options with pregnant women. Some evidence suggests that a recommendation from a health provider to have the vaccine increases the likelihood that women elect to receive the vaccine.[40] Women also raised concerns about the safety and the lack of effectiveness as two of the main reasons they chose not to receive the vaccine.[41] This is where well informed health professionals armed with the latest evidence on effectiveness and safety of the influenza vaccine can inform and educate women about the benefits of the vaccine, for both the pregnant woman and child under 6 months of age, as well as safety for both the mother and foetus.

Health professionals should be aware that the vaccine has been shown to be effective in reducing influenza illness and hospitalisation in infants up to six months of age. This should be conveyed to pregnant women as one of the key potential benefits of vaccination during pregnancy. Two-for-one benefit is an important piece of knowledge that influences future vaccination.[39]

4.12 Implications for recommendations and policy

New evidence gathered during the influenza A (H1N1) pandemic period has not necessitated a change to current recommendations or policy for the influenza vaccine, however it seems prudent to provide a prescriptive statement on the most effective and safest trimester to be vaccinated, in line with best available evidence. This may provide clinicians and pregnant women with clarity and confidence regarding the optimal timing of influenza vaccination in maximising effectiveness and safety.

It appears that vaccination during the second and third trimester should be the standard clinical practice. However, there are occasions that vaccination during the first trimester will need to be considered.

In Australia, UK, USA and Canada the influenza vaccine is recommended to be administered during any trimester of pregnancy.[40, 133-135] In Australia The Australian Immunisation Handbook 10th Edition recommends the following:[133]

"Recommended for all pregnant women at any stage of pregnancy, particularly those who will be in the second or third trimester during the influenza season."(P.135)[133]

The Australian Immunisation Handbook 10th Edition also states:

"Although it is recommended that all pregnant women should be immunised as early as possible in pregnancy, the precise timing of vaccination will depend on the time of the year, vaccine availability, influenza seasonality, gestation of pregnancy and the likely duration of immunity."(p.252)[133]

In the USA the recommendation refers to the second and third trimester in regards to the risk of harm from contracting the virus. They however recommend being vaccinated during any trimester with the key factor being the proximity of the influenza season. They recommend:

"Women in the second and third trimesters of pregnancy are at increased risk for hospitalisation from influenza. Because vaccinating against influenza before the season begins is critical, and because predicting exactly when the season will begin is impossible, routine influenza vaccination is recommended for all women who are or will be pregnant (in any trimester) during influenza season, which in the United States is usually early October through late March."[136]

In Canada the recommendation does not refer to the trimester of pregnancy and recommends any stage of pregnancy. They recommend:

"All pregnant women, at any stage of pregnancy, should be considered high priority for receiving inactivated influenza vaccine because of their increased risk of influenza-associated morbidity, evidence of adverse neonatal outcomes associated with maternal influenza, evidence that vaccination of pregnant women protects their newborns from influenza and influenza-related hospitalisation, and evidence that infants born during the influenza season to vaccinated women are less likely to be premature, small for gestational age, and low birth weight."[134]

In the UK they also do not refer to any trimester having better evidence than any other. They recommend:

"Pregnant women at any stage of pregnancy (first, second or third trimesters)." (p.199)[135]

Ambiguity and vagueness can reduce the adherence to recommendations, as well as lead to

inconsistent interpretation.[137] Expressing any issues regarding the safety of the vaccine may also decrease uptake of the vaccine, even though there is no evidence of any harm. In an effort to be clear, but also provide the clinician and pregnant women with the best evidence based statement regarding vaccination, it could read similar to this:

Influenza vaccination is recommended for pregnant women during any trimester of pregnancy. There is less evidence regarding the safety and effectiveness of the vaccine during the first trimester of pregnancy, although there is no evidence of harm. Pregnant women vaccinated during the second or third trimester can provide protection for their child up to 6 months of age.

4.13 Conclusion

Influenza vaccine administered during pregnancy is effective and provides a similar reduction in influenza-like illness as it does for a healthy adult population. Despite this, there is no evidence to assess the effectiveness of the influenza vaccine at reducing severe illness, or hospitalisation in pregnant women. Infants of pregnant women vaccinated during their second or third trimester can expect to have reduced rates of influenza, and influenza related hospitalisation, for their first 6 months of life.

Influenza vaccination during pregnancy had no harmful association with adverse outcomes for the foetus, including premature birth, small for gestational age, congenital malformation, spontaneous abortion, and foetal death. However, the evidence during the first trimester of pregnancy involved smaller numbers and requires further evaluation. Despite this, there are scenarios when vaccination during the first trimester of pregnancy will be appropriate. Serious adverse events requiring medical attention are rare for both pandemic and seasonal vaccines.

There is some evidence that influenza vaccination provides protection for the foetus during a pandemic influenza season and even during peak seasonal influenza periods. However, more experimental studies are needed to confirm these findings.

Despite a large body of new evidence, overall the results of this systematic review support the current recommendations regarding influenza vaccination during pregnancy.

Appendix I Search strategy

Pub med search

A	B	C
Influenza, human[mh] Influenza* [tiab] H1N1* [tiab] Flu [tiab]	Immunization [mh] Influenza Vaccines[mh] Influenza vaccin* [tiab] Immunization [tiab] Immunisation [tiab] Immunotherap* [tiab] vaccin* [tiab] immuni* [tiab] inocul* [tiab] efficacy [tiab] effectiveness [tiab] adverse* [tiab]	Pregnancy[mh] Infant, newborn [mh] Fetus [mh] Pregnan*[tiab] Maternal [tiab] Fetal [tiab] Fetus [tiab] Foet* [tiab] Infan* [tiab] Newborn* [tiab] Neonat*[tiab]

Result = 1864.

Embase

A	B	C
Influenza: exp Flu: ab,ti influenza*: ab, ti H1N1*: ab, ti	'influenza vaccine': exp 'swine influenza vaccine': de vaccin*: ab, ti immuni*: ab, ti safety: ab, ti effectiveness: ab, ti efficacy: ab, ti adverse: ab, ti innoculat*: ab, ti	Pregnancy: exp Infant: exp, ab, ti Fetus: exp, ab, ti Pregnan*: ab, ti Newborn:de, ab, ti Fetal: ab, ti Foetus: ab, ti Foetal: ab, ti Maternal: ab, ti

Result = 1749

Database	Search statement
Scopus	((TITLE-ABS-KEY(influenza* OR h1n1* OR flu) AND SUBJAREA(mult OR agri OR bioc OR immu OR neur OR phar)) AND (TITLE-ABS-KEY(pregnan* OR maternal OR fetal OR fetus OR foet* OR newborn* OR infant OR neonat*) AND SUBJAREA(mult OR agri OR bioc OR immu OR neur OR phar)) AND (TITLE-ABS-KEY("Influenza vaccin*" OR immunization OR immuni* OR vaccin* OR inocul*) AND SUBJAREA(mult OR agri OR bioc OR immu OR neur OR phar))) AND NOT (TITLE-ABS-KEY("Haemophilus influenzae") AND SUBJAREA(mult OR agri OR bioc OR immu OR neur OR phar)) AND (LIMIT-TO(EXACTKEYWORD, "Influenza vaccine") OR LIMIT-TO(EXACTKEYWORD, "Influenza")) AND (LIMIT-TO(LANGUAGE, "English")) AND (EXCLUDE(DOCTYPE, "re")) AND (EXCLUDE(SUBJAREA, "VETE")) Result = 520

Web of Science	#1 TS=("Influenza vaccin*" OR immunisation* OR immunization* OR vaccin* OR inocul*) #2 TS=(pregnan* OR maternal OR fetal OR fetus OR foet* OR newborn* OR neonat*) #3 TS=(influenza* OR flu OR H1N1*) NOT TI=Haemophilus influenzae (#1 AND #2 AND #3) AND Language=(English) AND Document Types=(Article OR Abstract of Published Item OR Book OR Book Chapter) Refined by: [excluding] Research Areas=(AGRICULTURE OR PARASITOLOGY OR VETERINARY SCIENCES OR ZOOLOGY) Timespan=All Years. Databases=SCI-EXPANDED, CPCI-S. Lemmatization=Off Result = 552
Scirus	((((Influenza OR (H1N1*)) (vaccin* OR immunisation OR immunization OR innocul*)) (pregnan* OR maternal OR fetal OR fetus OR foet* OR newborn* OR neonat*)) (experimental OR epidemiol* OR "controlled trial*" OR "clinical trial" OR prospective OR retrospective OR cohort OR "case control" OR "cross sectional")) ANDNOT (mice OR pig* OR rat* OR Haemophilus*) Result = 207
Mednar	Searched US department of Health and Human Services & World Health Organization Result = 197
ProQuest	influenza AND (pregnan* OR maternal) AND (immuni* OR vaccination) AND mesh.Exact("Influenza" OR "Vaccination" OR "Pregnancy") Age group Adolescent (13-18 years), Adult (19-44 years), Fetus (conception to birth), Infant (1-23 months), Newborn (birth to 1 month) Source type, Reports, Scholarly Journals, Document type, Article, Case Study, Conference, Conference Paper, Dissertation/Thesis, Statistics/Data Report. Language, English Result = 242
Trove	influenza AND pregnan* Fromat Thesis Result = 7

Appendix II Appraisal instruments

MAStARI Appraisal instrument

JBI Critical Appraisal Checklist for Randomised Control / Pseudo-randomised Trial

Reviewer _____ Date _____

Author _____ Year _____ Record Number _____

	Yes	No	Unclear	Not Applicable
1. Was the assignment to treatment groups truly random?	☐	☐	☐	☐
2. Were participants blinded to treatment allocation?	☐	☐	☐	☐
3. Was allocation to treatment groups concealed from the allocator?	☐	☐	☐	☐
4. Were the outcomes of people who withdrew described and included in the analysis?	☐	☐	☐	☐
5. Were those assessing outcomes blind to the treatment allocation?	☐	☐	☐	☐
6. Were the control and treatment groups comparable at entry?	☐	☐	☐	☐
7. Were groups treated identically other than for the named interventions	☐	☐	☐	☐
8. Were outcomes measured in the same way for all groups?	☐	☐	☐	☐
9. Were outcomes measured in a reliable way?	☐	☐	☐	☐
10. Was appropriate statistical analysis used?	☐	☐	☐	☐
	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info. ☐

Comments (Including reason for exclusion)

JBI Critical Appraisal Checklist for Comparable Cohort/ Case Control

Reviewer _____ Date _____

Author _____ Year _____ Record Number _____

	Yes	No	Unclear	Not Applicable
1. Is sample representative of patients in the population as a whole?	☐	☐	☐	☐
2. Are the patients at a similar point in the course of their condition/illness?	☐	☐	☐	☐
3. Has bias been minimised in relation to selection of cases and of controls?	☐	☐	☐	☐
4. Are confounding factors identified and strategies to deal with them stated?	☐	☐	☐	☐
5. Are outcomes assessed using objective criteria?	☐	☐	☐	☐
6. Was follow up carried out over a sufficient time period?	☐	☐	☐	☐
7. Were the outcomes of people who withdrew described and included in the analysis?	☐	☐	☐	☐
8. Were outcomes measured in a reliable way?	☐	☐	☐	☐
9. Was appropriate statistical analysis used?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info. ☐

Comments (Including reason for exclusion)

JBI Critical Appraisal Checklist for Descriptive / Case Series

Reviewer _____ Date _____

Author _____ Year _____ Record Number _____

	Yes	No	Unclear	Not Applicable
1. Was study based on a random or pseudo-random sample?	☐	☐	☐	☐
2. Were the criteria for inclusion in the sample clearly defined?	☐	☐	☐	☐
3. Were confounding factors identified and strategies to deal with them stated?	☐	☐	☐	☐
4. Were outcomes assessed using objective criteria?	☐	☐	☐	☐
5. If comparisons are being made, was there sufficient descriptions of the groups?	☐	☐	☐	☐
6. Was follow up carried out over a sufficient time period?	☐	☐	☐	☐
7. Were the outcomes of people who withdrew described and included in the analysis?	☐	☐	☐	☐
8. Were outcomes measured in a reliable way?	☐	☐	☐	☐
9. Was appropriate statistical analysis used?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

Appendix III: Data extraction instruments

MAStARI data extraction instrument

JBI Data Extraction Form for Experimental / Observational Studies

Reviewer _____ Date _____

Author _____ Year _____

Journal _____ Record Number _____

Study Method

RCT	☐	Quasi-RCT	☐	Longitudinal	☐
Retrospective	☐	Observational	☐	Other	☐

Participants

Setting

Population

Sample size

Group A _____ Group B _____

Interventions

Intervention A

Intervention B

Authors Conclusions:

Reviewers Conclusions:

Study results

Dichotomous data

Outcome	Intervention ( ) number / total number	Intervention ( ) number / total number

Continuous data

Outcome	Intervention ( ) number / total number	Intervention ( ) number / total number

Appendix IV: Excluded studies following retrieval of full text

Cheng AC, Kotsimbos T, Kelly HA, et al. Effectiveness of H1N1/09 monovalent and trivalent influenza vaccines against hospitalisation with laboratory-confirmed H1N1/09 influenza in Australia: a test-negative case control study. Vaccine 2011; 29(43): 7320-5.

Reason for exclusion: Pregnant women made up a small sub-population of this case control study. The effectiveness in population of interest for this review could not be analysed separately to the overall results.

Christian LM, Iams JD, Porter K, Glaser R. Inflammatory responses to trivalent influenza virus vaccine among pregnant women. Vaccine 2011; 29(48): 8982-7

Reason for exclusion: Inflammatory marker study, not outcome of interest

Dominguez A, Castilla J, Godoy P, et al. Effectiveness of pandemic and seasonal influenza vaccines in preventing pandemic influenza-associated hospitalisation. Vaccine 2012; 30(38): 5644-5650.

Reason for exclusion: The author was contacted and there was only 1 case and 0 controls in the sub-population of pregnant women in this study.

Fielding JE, Grant KA, Garcia K, Kelly HA. Effectiveness of seasonal influenza vaccine against pandemic (H1N1) 2009 virus, Australia, 2010. Emerg Infect Dis 2011; 17(7): 1181-1187

Reason for exclusion: Not population of interest.

Fielding JE, Grant KA, Tran T, Kelly HA. Moderate influenza vaccine effectiveness in Victoria, Australia, 2011. Euro Surveill 2012; 17(11).

Reason for exclusion: Not population of interest.

Fisher BM, Scott J, Gibbs RS, Lynch A, Winn VD, Weinberg A. Antibody responses to pandemic and seasonal influenza A (H1N1) strains during the 2009-2010 influenza season: Are the responses during pregnancy similar? Am J Obstet Gynecol 2012; 206(1): S264

Reason for exclusion: Immunologic study, not outcome of interest

Fisher BM, Van Bockern J, Hart, J, Lynch AM, Winn VD, Gibbs RS, Weinberg A. Pandemic influenza A (H1N1) 2009 infection versus vaccination: a cohort study comparing immune responses in pregnancy. PLoS One 2012; 7(3): e33048.

Reason for exclusion: Immunologic study, not outcome of interest

Folkenberg M, Callréus T, Svanström H, Valentiner-Branth P, Hviid A. Spontaneous reporting of adverse events following immunisation against pandemic influenza in Denmark November 2009-March 2010. Vaccine 2011; 29(6): 1180-4

Reason for exclusion: Study using passive surveillance. Methodology not suitable for this review.

Forbes RL, Wark PB, Murphy VE, Gibson PG. Pregnant women have attenuated innate interferon responses to 2009 pandemic influenza a virus subtype (H1N1). J Infec Dis 2012; 206(5): 646-653.

Reason for exclusion: Immunologic study, not outcome of interest

France EK, Smith-Ray R, McClure D, et al. Impact of maternal influenza vaccination during pregnancy

on the incidence of acute respiratory illness visits among infants. Arch Pediatr Adolesc Med 2006; 160(12): 1277-83.

Reason for exclusion: Infants were followed up to at least 11 months after birth. No specific data is available for children under 6 months of age as per the requirements of this protocol.

Gilman EA, Wilson LM, Kneale GW, Waterhouse JA. Childhood cancers and their association with pregnancy drugs and illnesses. Paediatr Perinat Epidemiol 1989; 3(1): 66-94.

Reason for exclusion: No outcome measures for influenza vaccination. Not outcome of interest

Griffin MR, Monto AS, Belongia EA, et al. Effectiveness of non-adjuvanted pandemic influenza a vaccines for preventing pandemic influenza acute respiratory illness visits in 4 U.S. communities. PLoS One 2011; 6(8).

Reason for exclusion: Pregnant women are not identified as a sub-population in this study. Not population of interest.

Hardelid P, Fleming DM, McMenamin J, et al. Effectiveness of pandemic and seasonal influenza vaccine in preventing pandemic influenza A (H1N1) 2009 infection in England and Scotland 2009-2010. 2011 Euro Surveill 16(2).

Reason for exclusion: Pregnant women are not identified as a sub-population in this study. Not population of interest.

Huang WT, Chen WC, Teng HJ, et al. Adverse events following pandemic A (H1N1) 2009 monovalent vaccines in pregnant women - Taiwan, November 2009-August 2010. PloS One 2011; 6(8).

Reason for exclusion: Study using passive surveillance and case capture methodology. Methodology not suitable for this review.

Jimenez-Jorge S, de Mateo S, Pozo F, et al. Early estimates of the effectiveness of the 2011/12 influenza vaccine in the population targeted for vaccination in Spain, 25 December 2011 to 19 February 2012. Euro Surveill 2012; 17(12).

Reason for exclusion: Pregnant women made up 5 cases of the study and 1 test negative control. Not population of interest.

Jimenez-Jorge S, Savulescu C, Pozo F, et al. Effectiveness of the 2010-11 seasonal trivalent influenza vaccine in Spain: CycEVA study. Vaccine 2012; 30(24): 3595-3602.

Reason for exclusion: Pregnant women made up 1 case of the study and 5 test negative control. Not population of interest.

Kankawinpong O, Sangsajja C, Cholapand A, et al. Immunogenicity and safety of an inactivate pandemic (H1N1) vaccine provided by the Thai ministry of public health as a routine public health service. Southeast Asian J Trop Med Public Health 2012; 43(3): 680.

Reason for exclusion: Pregnant women made 2% of the study population. No specific safety results were available for pregnant women.

Kelly, H. Carville, K. Grant, K. Jacoby, P. Tran, T. Barr, I. Estimation of influenza vaccine effectiveness from routine surveillance data. PLoS One 2009; 4(3).

Reason for exclusion: Not population of interest.

Kelly HA, Grant KA, Fielding JE, et al. Pandemic influenza (H1N1) 2009 infection in Victoria, Australia: No evidence for harm or benefit following receipt of seasonal influenza vaccine in 2009. Vaccine 2011; 29(37): 6419-6426.

Reason for exclusion: Not population of interest.

Kissling E, Valenciano M, Cohen JM, et al. I-MOVE multi-centre case control study 2010-11: Overall and stratified estimates of influenza vaccine effectiveness in Europe. PLoS One 2011; 6(11).

Reason for exclusion: Not population of interest.

Kissling E, Valenciano M. Early estimates of seasonal influenza vaccine effectiveness in Europe, 2010/11: I-MOVE, a multicenter case-control study. Euro Surveill 2011; 16(11).

Reason for exclusion: Not population of interest.

Kissling E, Valenciano M. Early estimates of seasonal influenza vaccine effectiveness in Europe among target groups for vaccination: Results from the I-MOVE multicenter case-control study, 2011/12. Euro Surveill 2012; 17(15).

Reason for exclusion: Not population of interest.

Mahmud SM, van Caeseele P, Hammond G, Kurbis C, Hilderman T, Elliott L. No association between 2008-09 influenza vaccine and influenza A (H1N1)pdm09 virus infection, Manitoba, Canada, 2009. Emerg Infect Dis 2012; 18(5): 801-810.

Reason for exclusion: 127 pregnant women were included in the study population. No separate effectiveness results were available for pregnant women.

Moro PL, Broder K, Zheteyeva Y, et al. Adverse events in pregnant women following administration of trivalent inactivated influenza vaccine and live attenuated influenza vaccine in the Vaccine Adverse Event Reporting System, 1990-2009. Am J Obstet Gynecol 2011; 204(2): 146.e1-146.e7.

Reason for exclusion: Report on passive surveillance, methodology not suitable for this review.

Moro PL, Broder K, Zheteyeva Y, et al. Adverse events following administration to pregnant women of influenza A (H1N1) 2009 monovalent vaccine reported to the Vaccine Adverse Event Reporting System. Am J Obstet Gynecol.2011; 205(5): 473.e1-473.e9.

Reason for exclusion: Passive surveillance, methodology not suitable for this review.

Orozova-Bekkevold I, Jensen H, Stensballe L, Olsen J. Maternal vaccination and preterm birth: using data mining as a screening tool. Pharm World Sci 2007; 29(3): 205-212.

Reason for exclusion: No specific analysis or results on influenza vaccination available. Not outcome of interest.

Ortqvist A, Berggren I, Insulander M, De Jong B, Svenungsson B, Effectiveness of an adjuvanted monovalent vaccine against the 2009 pandemic strain of influenza A (H1N1)v, in Stockholm county, Sweden. Clin Infect Dis 2011; 52(10): 1203-1211.

Reason for exclusion: Pregnant women make up a sub population of this study. The results and data are unable to be separated from the entire study population.

Savulescu C, Jimenez-Jorge S, de Mateo S, et al. Using surveillance data to estimate pandemic vaccine effectiveness against laboratory confirmed influenza A (H1N1) 2009 infection: two case-control studies, Spain, season 2009-2010. BMC Public Health 2011; 11; 899.

Reason for exclusion: Pregnant women made up 5 cases of the study and 7 test negative controls. No specific results were available for pregnant women.

Simpson CR, Ritchie LD, Robertson C, Sheikh A, McMenamin J. Effectiveness of H1N1 vaccine for the prevention of pandemic influenza in Scotland, UK: a retrospective observational cohort study. Lancet Infect Dis 2012; 12(9): 696-702.

Reason for exclusion: author contacted, unable to provide a sub group analysis on the pregnant women vaccinated.

Sonmezer M, Tuncer Ertem G, Ucal Bakkal S, Bulut C, Kinikli S, Tulek N. The side effects of H1N1 pandemic vaccine in pregnant women and comparison to other healthcare workers. Clin Microbiol Infec 2011; 17: S830.

Reason for exclusion: abstract only available.

Sperling RS, Engel SM, Wallenstein S, et al. Immunogenicity of trivalent inactivated influenza vaccination received during pregnancy or postpartum. Obstet Gynecol 2012; 119(3): 631-9.

Reason for exclusion: Immunologic study, not outcome of interest.

Steens A, Wijnans E, Dieleman J, et al. Effectiveness of a MF-59(trademark)-adjuvanted pandemic influenza vaccine to prevent 2009 A/H1N1 influenza-related hospitalisation; a matched case-control study. BMC Infect Dis 2011; 11.

Reason for exclusion: Not population of interest

Steinhoff MC, Omer SB, Roy E, et al. Influenza Immunization in Pregnancy - Antibody Responses in Mothers and Infants. N Eng J Med 2010; 362(17): 1644-1646.

Reason for exclusion: Immunologic study, no outcome of interest.

Tsai T, Kyaw MH, Novicki D, Nacci P, Rai S, Clemens R. Exposure to MF59-adjuvanted influenza vaccines during pregnancy - A retrospective analysis. Vaccine 2010; 28(7): 1877-1880.

Reason for exclusion: Secondary analysis of data included in other studies in the review.

Yates L, Pierce M, Stephens S, et al. Influenza A/H1N1v in pregnancy: an investigation of the characteristics and management of affected women and the relationship to pregnancy outcomes for mother and infant. Health Technol Assess 2010; 14(34): 109-82.

Reason for exclusion: No outcome of interest.

Excluded following critical appraisal.

Heinonen OP, Shapiro S, Monson RR, Hartz SC, Rosenberg L, Slone D. Immunization during pregnancy against poliomyelitis and influenza in relation to childhood malignancy. Int J Epidemiol 1973; 2(3): 229-35.

Reason for exclusion: <5 criteria. The paper primarily looks at Killed Polio Vaccine. The methodology and data behind the influenza arm of the study is unclear. The one case with a neurological disorder also had the polio vaccine.

Murray DL, Imagawa DT, Okada DM, St Geme JWJr. Antibody response to monovalent A/New Jersey/8/76 influenza vaccine in pregnant women. J Clin Microbiol 1979; 10(2): 184-7.

Reason for exclusion: <5 criteria. Adverse events methodology not described.

Ohfuji S, Fukushima W, Deguchi M, et al. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine among pregnant women: Lowered antibody response by prior seasonal vaccination. J Infect Dis 2011; 203(9): 1301-1308.

Reason for exclusion: <5 criteria. Primarily an Immunogenicity study. No methodology of adverse event deliberation or follow up. Unclear if birth outcomes were followed up. Attrition of 25 women at the point of the second vaccination.

Yamada T, Morikawa M, Cho K, et al. Pandemic (H1N1) 2009 in pregnant Japanese women in Hokkaido. J Obstet Gynaecol Res 2012; 38(1): 130-6.

Reason for exclusion: <5 criteria. Unclear how influenza was diagnosed. Potential for confounding with antiviral treatments.

Zuccotti G, Pogliani L, Pariani E, Amendola A, Zanetti A. Transplacental antibody transfer following maternal immunization with a pandemic 2009 influenza A(h1n1) mf59-adjuvanted vaccine. JAMA 2010; 304(21): 2360-2361.

Reason for exclusion: <5 criteria. Unclear methodology with adverse events

Appendix V: Included studies

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Benowitz et al. (2010)[84]	**Study design:** Case control study **Statistical methods:** Effectiveness of the vaccine was calculated as 1 minus the matched odds ratio of both cohorts X 100. Logistic regression was performed for multiple potential confounders. Their adjusted model retained, vaccination of household contacts, and prematurity. Risk set sampling was used to match controls that were admitted with influenza-like illness, but negative for influenza.	**Setting:** Large metropolitan hospital in USA **Participants:** Infants aged <12 months (only children < 6 months included). 11.6% of group 1 and 19.3% of group 2 were born premature. 36.3% of group 1 and 38.5% of group 2 had chronic conditions. **Trimester vaccinated:** 2nd or 3rd. 78% in the 3rd trimester.	Primary intervention trivalent seasonal vaccination during pregnancy. **Group 1)** Infants hospitalised with positive influenza direct fluorescent antibody test. (n=91) **Group 2)** Infants hospitalised with negative influenza direct fluorescent antibody test. (n=156)	**Aim:** Assess the effectiveness of influenza vaccine given to pregnant women in decreasing the number of hospitalisations for laboratory-documented influenza among their infants. **Study period:** Putative season between October and April 2000-09. Finished prior to (H1N1) 2009 pandemic. No mention of strains or match.	Hospitalisation of infants up to 6 months	Adjusted 91.5% (95% CI: 61.7% to 98.1%)	Interviews with parents were conducted as well as review of medical records.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Black et al. (2004)[7]	**Study design:** Retrospective cohort study. **Statistical methods:** Cox proportional hazards regression was used to investigate effectiveness and adjusted for women's age and week of delivery. Effectiveness outcomes for infants were adjusted for gender, gestational age, week of birth, and birth facility.	**Setting:** Northern California health service, USA **Participants:** Pregnant women: Contained all women with live births Infants: Contained all live births for the same period Characteristics of participants and maternal risk factors not described. **Trimester vaccinated:** Not stated	**Group 1)** Seasonal Influenza vaccination. Type not reported. Pregnant women: (n=3707) Infants: (n=3652) **Group 2)** No influenza vaccination during pregnancy Pregnant women: (n=45878) Infants: (n=44987)	**Aim:** Evaluate the impact of influenza vaccination during pregnancy on women and their risk of influenza illness, as well as its effect on the risk of influenza-like illness in infants during the flu season. **Study period:** Influenza seasons from 1997 to 2002 defined by the first and last virus isolates. Unclear of circulating strains or match of vaccine.	Preterm delivery (< 37 weeks) Outpatient visit for pregnant women with an influenza-like symptom Hospital admission of infants with pneumonia Outpatient visit for infants with an influenza like illness	Vaccinated (7.37%) compared with no vaccination (6.72%) (p=0.136). Adjusted HR 1.51 (p=0.088). When presentations of asthma were excluded HR 1.001 (p=0.988) Excluded not restricted to < 6 months. Excluded not restricted to < 6 months.	Data obtained using medical diagnostic coding. Unclear population details and limited assessment of potential confounding factors.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Candela et al. (2012)[53]	**Study design:** Prospective single arm study **Statistical methods:** Data were presented as frequencies and percentages.	**Setting:** Local health district in Italy **Participants:** Characteristics of pregnant women and maternal risk factors not described. **Trimester vaccinated:** 2nd or 3rd trimester. Exact numbers not specified.	**Group 1)** Influenza A (H1N1) 2009 vaccine. MF59 adjuvanted subunit vaccine Mothers (n=370) Infants born during the study period (n=70)	**Aim:** Evaluating safety in real time and assess effectiveness and long-term safety using record linkage between regional databases. **Study period:** Between October 2009 and January 2010	Adverse events pregnant women Serious adverse events Adverse events of special interest Premature birth	41 pregnant women reported 26 medically confirmed adverse events and 33 self-reported adverse events. Most frequently reported were common cold (11.5%), cough (11.5%), diarrhoea and gastroenteritis (11.5%). 7 serious adverse events were reported. None were suspected to be vaccine related. No adverse events of special interest occurred. 3 births.	Pregnant women made up a sub-population of a larger cohort. Active surveillance was conducted by telephone and physicians were contacted for adverse event confirmation and clinical information. Low power for assessing uncommon adverse events
Conlin et al. (2013)[81]	**Study design:** Retrospective cohort study **Statistical methods:** Data were presented as frequencies and	**Setting:** U.S. military **Participants:** Pregnant service women. 92.5%	**Group 1)** Influenza A (H1N1) 2009 vaccine. Adjuvants not stated. 64.4%	**Aim:** Assess adverse pregnancy outcomes among active-duty U.S.	Pregnancy loss Preeclampsia or eclampsia,	6.4% (H1N1) vaccinated group, 6.5% seasonal group 5.8% (H1N1) vaccinated group 5.2%seasonal	ICD-9 coding from the Military Health System Data Repository was used to

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	percentages for the outcomes in this review.	of group 1 participants were under 35 and 92.7% in group 2. **Trimester vaccinated:** All trimesters, 4122 (39.7%) during 1st trimester for (H1N1) 2009 and 2745 (36.3%) in the seasonal group.	also had seasonal influenza vaccine Mothers: (n=10376) Infants: (n=9435) **Group 2)** Seasonal influenza A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2)-like virus, and B/Florida/4/2006, 2008/2009 vaccine Mothers: (n=7560) Infants: (n=6759)	military women who received pandemic (H1N1) vaccine during pregnancy as well as adverse health outcomes among the newborns resulting from these pregnancies. **Study period:** October 1, 2009, and June 30, 2010. October 1, 2008, and June 30, 2009. Aligned with vaccination timing.	Preterm labour as % of vaccinated cohorts. Congenital malformation (Definition from the National Birth Defects Prevention Network using ICD-9 coding for congenital abnormalities.)	vaccinated group 6.5% (H1N1) vaccinated group, 6.2% seasonal vaccinated group 2.1% (H1N1) vaccinated group, 2.0% seasonal vaccinated group	identify pregnancy related codes. Only maternal risk factors described were age and African ancestry
Cristiani et al. (2011)[54]	**Study design:** Prospective cohort study **Statistical methods:**	**Setting:** Health district in Italy **Participants:**	**Group 1)** Influenza A (H1N1) 2009 MF59	**Aim:** Determine the frequencies and clinical features	Adverse events pregnant women	11 adverse events were reported in 7 pregnant women, 4 headache, 4 injection	Pregnant women made up a small sub-population of

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	Descriptive outcomes only considered for this review.	Pregnant women. Median age 33 years (range 25–39 years). Comorbidities were not reported. **Trimester vaccinated:** 2nd or 3rd trimester. Median gestational age at vaccination 24 weeks (range 15–36 weeks)	adjuvanted subunit vaccine (n=3) **Group 2)** Both seasonal influenza A/Brisbane/59/2007(H1N1)-like strain, (A/Brisbane/59/2007 IVR-148), A/Brisbane/10/2007(H3N2)-like strain, (A/Uruguay/716/2007 NYMC X-175C), B/Brisbane/60/2008-like strain (B/Brisbane/60/2008) and inactivated influenza (H1N1) 2009. (n=10)	of adverse events	Preterm birth	site reaction, 2 fatigue, and 1 respiratory tract infection. 1 at 35 weeks gestation	this case control study. An interview was conducted at the time of vaccination, and then telephone interviews were conducted at day 7, month 1, month 2, month 4, and month 6. Other than age no maternal risk factors were described

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Deinard AS, Ogburn PJr. (1981)[78]	**Study design:** Prospective cohort study **Statistical methods:** Data were presented as frequencies and percentages. Chi-square analysis was performed for congenital abnormalities.	**Setting:** Obstetric clinic in USA **Participants:** Pregnant women. Age ranged from 14 to 45 years, mean 21.97 (SD±4.6). 15.3% had a chronic illness in the vaccinated cohort, 13.9% in the unvaccinated. **Trimester vaccinated:** All trimesters, 41 (23%) were vaccinated in the first trimester. 33% in 2nd and 44% in 3rd.	**Group 1)** Either split or whole virus monovalent influenza A/New Jersey/8/76 virus vaccine Hsw1N1. (n=176) excludes 13 pre conception vaccinations **Group 2)** No influenza vaccination during pregnancy (n=517)	**Aim** Not stated **Study period:** 1976 to 1977, exact period unclear. The circulating strains and the match of the vaccine is also unclear.	Systemic reactions Serious adverse events Spontaneous abortion (<20 weeks gestation) Stillbirth (>20 weeks gestation) Live premature birth (<37 weeks) Congenital malformation (Medically diagnosed major and minor malformations)	Arthralgia or myalgia 2.7%, headache 1.6%, fever 37.8- 38.6 1.2%, fever >38.6 0.1%, malaise 1.1%, chills 0.6%, and cough 0.2%. No major or life-threatening reactions 2 in vaccinated group, 6 in unvaccinated group 1 in each cohort 16 (9.1%) vaccinated 55 (10.6%) unvaccinated 14 (8.0%) vaccinated 67 (13.0%) unvaccinated, including 13 pre-conception vaccinations (p <0.005)	Information was collected at the time of vaccination and then active surveillance was conducted. Information was also obtained on the infant's health up to the end 8 weeks after birth. 13.4% of participants were lost to follow up. Maternal risk factors described included race, chronic illness, and smoking.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Eick et al. (2011)[37]	**Study design:** Prospective cohort study **Statistical methods:** Relative risks were calculated. Potential confounders were investigated; day-care attendance, household smoking, wood or coal burning stove in the home, and breastfeeding. No statistically significant associations were found and no adjustments were made.	**Setting:** Apache Indian reservation in Arizona, USA **Participants:** Navajo and White Mountain Apache Indian mother-infant pairs. Infants were male 51% and female 49%. Other infant characteristics not described. **Trimester vaccinated:** 2nd or 3rd trimester. Exact numbers not described other than those with cord blood sample.	**Group 1)** Inactivated seasonal influenza vaccine strains B/HK, A/New Cal (H1N1), A/Panama (H3N2), A/Wyoming (H3N2), B/Shanghai. Thiomersal-reduced vaccine. Infants and mothers (n=573) **Group 2)** No influenza vaccination during pregnancy Infants and mothers (n=587)	**Aim:** Assess the effect of seasonal influenza vaccination during pregnancy on laboratory-confirmed influenza in infants to 6 months of age. **Study period:** 3 influenza seasons from November 2002 to September 2005. Not clear how these periods were chosen.	Influenza-like illness requiring hospitalisation, with at least one of the following: fever > 38.0°C, diarrhoea, or respiratory symptoms infants < 6 months. Influenza confirmed by virus culture, serology, or rapid influenza diagnostic test infants < 6 months Influenza-like illness medical visit with at least one of the following: fever > 38.0°C, diarrhoea, or	RR 0.61 (95% CI: 0.45 to 0.84), RR 0.59 (95% CI: 0.37 to 0.93) RR 0.92 (95% CI: 0.73 to 1.16)	Active surveillance was conducted until the child reached 6 months of age. The study was done on a vulnerable population and may not be generalisable to other populations. Outcomes for the people who withdrew are unclear. Statistical difference in breastfed infants with 74% in unvaccinated cohort and 81% in vaccinated cohort

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
					respiratory symptoms.		(p=0.007)
Englund et al. (1993)[55]	**Study design:** Pseudo randomised controlled trial **Statistical methods:** Descriptive outcomes only considered for this review.	**Setting:** Family practice in USA **Participants:** Healthy pregnant women. No other description of characteristics. **Trimester vaccinated:** 3rd trimester	**Group 1)** Trivalent A/Sichuan/ H3N2, A/Taiwan/HINI and B/Victoria influenza vaccination. (n=13) **Group 2)** Tetanus toxoid vaccination. (n=13)	**Aims:** Not stated. Primarily an immunologic study	Adverse events	No significant adverse reaction including fever, moderate or severe pain, or need to visit a physician were reported.	The method of data collection for adverse events was unclear. Small sample size. Unclear randomisation and concealment.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Fell et al. (2012)[85]	**Study design:** Retrospective cohort **Statistical methods:** Logistic regression was used to compare risks for each outcome between the exposure groups. Multivariable regression was performed to calculate adjusted risk ratios. Confounders considered included maternal age, parity, history of premature birth, smoking during pregnancy, chronic hypertension, medical comorbidity, pregnancy-induced hypertension, preeclampsia, obstetrical complications, intrapartum complications during labour or birth, and socioeconomic status. All adjusted models included maternal age,	**Setting:** Ontario residents in Canada **Participants:** All singleton hospital births (20 weeks gestation or more and birth weight equal to or greater than 500 g). Mothers maternal age less than 35 (78%), medical comorbidity 7.4%. **Trimester vaccinated:** Unknown	**Group 1)** Influenza A (H1N1) 2009 vaccine alone. Adjuvant or type of vaccine not stated. (n=21363), Some also had the seasonal vaccine: (n=1977) Total (n=23340) **Group 2)** Not vaccinated with either influenza A (H1N1) 2009 or seasonal trivalent vaccination. (n=32230)	**Aims:** Evaluate the relationship between maternal H1N1 vaccination and foetal and neonatal outcomes during the 2009–2010 (H1N1) pandemic. **Study period:** The study period was November 2009 to April 2010, the authors reported the match between the vaccine and circulating was "very strong". No information was reported regarding the selection of the study period.	Premature birth (<37 weeks) Very premature birth (<32 weeks) SGA birth (below the 10th percentile) Severe SGA (below the 3rd percentile)	Adjusted RR 0.95 (95% CI: 0.88 to 1.02) Adjusted RR 0.73 (95% CI: 0.58 to 0.91) Premature birth measures also adjusted for chronic hypertension, pregnancy-induced hypertension, preeclampsia, history of preterm birth, and maternal smoking. Adjusted RR 0.90 (95% CI: 0.85 to 0.96) Adjusted RR 0.81 (95% CI: 0.72 to 0.92) SGA also adjusted for neighbourhood immigrant concentration, chronic hypertension, and maternal smoking.	Data was obtained from Ontario's electronic birth record. Socioeconomic data was obtained from the 2006 Canadian census. Trimester of vaccination not able to be reported. Maternal risk factors described as per variables in regression model.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	family income, and education. Among live births only.				Foetal death (intrauterine death greater than 20 weeks gestation)	Adjusted RR 0.66 (95% CI: 0.47 to 0.91) Foetal death also adjusted for maternal smoking	
Hårmark et al. (2011)[86]	**Study design:** Single arm prospective study. **Statistical methods:** Multivariate logistic regression was performed. The model compared gender, age (0–52.5 years), (52.5–61.9 years), (61.9–67.2 years), (67.2–90.0 years), cardiovascular disease, pulmonary disease, immunodeficiency, and pregnancy. Chi-square test was also performed.	**Setting:** GP practices in the Netherlands **Participants:** Group 1) Pregnant women. Characteristics not described. Group 2) all participants with a medical indication that warranted the seasonal flu vaccination. **Trimester vaccinated:** 2nd or 3rd trimester.	**Group 1)** Vaccination with influenza A (H1N1) 2009 MF-59 adjuvanted subunit vaccine, two doses at least 2 weeks apart. (n=72) **Group 2)** Comparison with other risk groups enrolled in the study with the same intervention. (n=3703) 84% of the	**Aim:** Identify and quantify adverse events associated with a specific pandemic vaccine. Secondly investigate risk factors for the occurrence of adverse events. **Study period:** November 2009 to March 2010.	Risk of adverse event following influenza vaccination, during pregnancy	OR 2.61 (95% CI: 1.55 to 4.40, p=0.001)	Participants received a questionnaire via e-mail at 1 week, then following 2nd vaccination and then at 3 months. Attrition of 615 participants. It is unclear how many were from the pregnancy sub-group. Small sample size.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
			total participants also received the seasonal flu vaccination a few weeks earlier.				
Heikkinen et al. (2012)[87]	**Study design:** Mixed prospective and retrospective cohort study **Statistical methods:** Logistic regression and proportional hazard models were used with the latter using gestational age as the time factor for all outcomes except congenital malformations. Models adjusted for parity, smoking, and maternal age.	**Setting:** Midwife, hospital, and GP settings in Argentina, Italy, and Netherlands. **Participants:** Healthy pregnant women. Overall mean age 31.6 years **Trimester vaccinated:** Any trimester, 94 (4%) in the 1st trimester. 1319 (56.9%) in the 2nd and 889 (38.7%) in	**Group 1)** Monovalent Influenza A (H1N1) 2009, MF59 adjuvanted subunit vaccine. 75% received 2 doses. (n=2295). **Group 2)** Women not vaccinated with influenza vaccine. (n=2213). Infants followed up at 3 months of	**Aim:** Evaluate outcomes in pregnant women who received the MF59 adjuvanted A (H1N1) influenza vaccine during the recent pandemic. **Study period:** November and December 2009 in the Netherlands, October and December 2009 in Italy, February and August 2010 in	Premature birth Foetal death after 22 weeks gestation. Spontaneous abortion prior to 22 weeks gestation.	Adjusted OR 0.75 (95% CI: 0.55 to 1.01), adjusted HR 0.69 (95% CI: 0.51 to 0.92) Adjusted OR 1.44 (95% CI: 0.23 to 8.90) and HR 1.38 (95% CI: 0.22 to 8.47) No case was reported in the vaccinated cohort versus 9 cases (0.4%) in the unvaccinated cohort. The denominator of women that received the vaccine prior to 22 weeks is unclear. There were 94 women vaccinated in their first trimester.	Information was gathered prospectively during antenatal visits. Another group was enrolled at delivery and information was collected from medical records. Attrition of 153 women with 16 dropping out of the study and 137 live births lost to follow up. Low numbers

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		the 3rd. 8 were missing.	age (n=4385)	Argentina. Unclear on rationale of study period timing or overlap with peak influenza period.	All congenital malformations defined as such in EUROCAT guidelines. Preeclampsia Low birth weight Maternal death	Adjusted OR 1.33 (95% CI: 0.88-2.00). Rates per trimester vaccinated; first trimester (2 of 94, 2.1%), second (35 of 1319, 2.7%), third (19 of 889, 2.1%). Adjusted OR 1.12 (95% CI: 0.81 to 1.55). HR 1.10 (95% CI: 0.80 to 1.53) Adjusted 0.88 (95% CI: 0.61 to 1.26). No maternal deaths or abortions occurred among the vaccinated women.	of women were vaccinated in their first trimester. Maternal risk factors described included age, race, smoking, alcohol consumption, and drug use.
Horiya et al. (2011)[88]	**Study design:** Prospective cohort study **Statistical methods:** Data were presented as frequencies and percentages.	**Setting:** Unclear, Japan **Participants:** Healthy pregnant women, mean age 34.8 (SD±4.1) years in group	**Group 1)** 2 doses of split-virion influenza A (H1N1) 2009 vaccine containing neither an adjuvant nor	**Aim:** Evaluate the efficacy of double vaccination with the 2009 pandemic influenza A (H1N1) vaccine	Adverse events.	Redness at the vaccination site was the most common reaction, then pain and induration. Less common were systemic symptoms; headache, malaise, fever, and nausea.	The methodology is unclear as to how adverse events were followed up and assessed.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		1 and 35.7 (SD±3.6) years group 2. **Trimester vaccinated:** Any trimester, 15 in the 1st trimester. 79 in the 2nd and 29 in the 3rd. 3 trimesters of vaccination not reported.	preservative (n=128) **Group 2)** 1 dose of same vaccine as group 1. (n=82)	during pregnancy. **Study period:** October 2009	Serious adverse events Premature birth Foetal death (No definition described. It is unclear what gestational ages were included.) Congenital abnormalities (No definition)	Overall incidence of adverse reactions was less than 10%. No serious adverse events requiring medical intervention were reported. 7% in participants vaccinated in the first or third trimester and 2% in those vaccinated in the second trimester. One aborted 5-month twin gestation 40 days after immunisation. 5 (4.2%) born to women vaccinated in their 2nd and 3rd trimester. No women vaccinated during 1st trimester gave birth to a child with a congenital abnormality.	

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Hulka (1964)[89]	**Study design:** Mixed prospective and retrospective cohort study **Statistical method** Data were presented as frequencies and percentages. The authors reported statistical significance, but there was no method reported.	**Setting:** Obstetric department in USA **Participants:** Pregnant women of low socioeconomic background, largely African American population. Other characteristics not described. **Trimester vaccinated:** Any trimester, 19 (5.2%) in the 1st trimester. Numbers not reported for 2nd and 3rd trimester.	**Group 1)** Polyvalent inactivated whole-virion influenza vaccine. Unclear as to antigenic makeup. (n=363) **Group 2)** Pregnant women vaccinated with placebo (n=181)	**Aim** Not directly stated. Investigate the beneficial effect of vaccination in pregnant women. **Study period:** October 1962 to January 1963. Circulating strain was identified; it is unclear if this matched the vaccine. Putative period used.	Local adverse events Systemic adverse events Influenza-like illness of pregnant women (self-reported respiratory ailment with fever) Congenital malformation (no definition)	40% of patients receiving saline complained of pain and 83% of vaccinated reported pain. 43% potentially attributable to the vaccine. 15-20% vaccinated and 1.9% unvaccinated pregnant women experienced malaise. This was reported as being statistically significant. 24 (11%) vaccinated and 36 (20%) unvaccinated experienced a fever and upper respiratory disease. No congenital anomalies in the 13 women who were vaccinated in their first trimester and followed up.	It is unclear how each cohort were allocated. Not blinded, allocation of treatment was not concealed; outcomes for people that withdrew were not included. Large attrition rate (n=149). Some women were added retrospectively to the control group if they were not vaccinated during the previous winter.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Hwang et al. (2011)[90]	**Study design:** Single arm prospective survey. **Statistical methods:** Local and systemic adverse event frequencies were compared according to other potential risk factors; sex, age, BMI, smoking, regular alcohol consumption, and comorbidity, by using the chi-squared test or Fisher's exact test. Logistic regression model used local or systemic adverse event as the dependent variable and age < 20, 20-29, 30-39, 40-49>50 years, BMI, smoking, regular alcohol consumption, comorbidity, and pregnancy as the independent variables.	**Setting:** Military healthcare Korea **Participants:** Sub population of 21 pregnant women. Characteristics of pregnant women not described. **Trimester vaccinated:** Unclear.	Group 1) Influenza A (H1N1) 2009 split-virion non-adjuvant vaccine. Pregnant women. (n=21) Group 2) Other risk groups enrolled in the study with the same intervention as group 1. (n=875)	**Aim:** Evaluates the incidence of and the risk factors for adverse events to influenza A (H1N1) **Study period:** October to December 2009.	Local reaction. Odds of local reaction in pregnant women compared to other risk groups. Systemic reaction. Odds of a systemic reaction in pregnant women compared to other risk groups.	38.1% experienced a local reaction. Adjusted OR 1.82 (95% CI: 0.72 to 4.56) 33.1% experienced a systemic reaction Adjusted OR 0.95 (95% CI: 0.37 to 2.45).	Active surveillance methods were used, as well as passive surveillance from military doctors. Low statistical power with only 21 vaccinated pregnant women enrolled.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Håberg et al. (2013)[18]	**Study design:** Retrospective cohort study **Statistical methods:** Cox proportional hazard model was used with gestational day as the underlying time metric. One model used clinical diagnosis of influenza during pregnancy with the following categories: no exposure to the pandemic (reference), exposure without a clinical diagnosis, and exposure with a clinical diagnosis. Another model was pregnancy days during the main pandemic wave and the end point was physician consultation for influenza.	**Setting:** Whole of pregnant population Norway **Participants:** Pregnant women with singleton pregnancies. 80% were aged less than 35 and 11% had a chronic illness. **Trimester vaccinated:** All trimesters, 2431 (9.4%) in the 1st trimester, 10827 (41.7%) in the 2nd,and 12718 (49.0%) in the 3rd trimester.	**Group 1)** Influenza A (H1N1) 2009 split-virion with ASO3 adjuvant. (n=25976) 266 women received 2 doses. **Group 2)** Not vaccinated with influenza vaccine. (n=87335)	**Aim:** Assess the effectiveness of the pandemic vaccine in pregnant women and the effect of vaccination or influenza on foetal survival **Study period:** October 1, 2009, and December 31, 2009. Period identified by the start and end of peak influenza period.	Risk of foetal death (After 12 weeks of pregnancy) The risk of premature birth following vaccination, (<37 weeks) The risk of term low birth weight (>37 weeks <2500g) The risk of an influenza	Adjusted HR 0.88 (95% CI: 0.66 to 1.17). Adjusted HR 1.00 (95% CI: 0.93 to 1.09) Adjusted HR 0.90 (95% CI: 0.76 to 1.08). All of the above HR were adjusted for influenza exposure and vaccination status, age, parity, marital status, use of nutritional supplements during pregnancy, smoking during pregnancy, history of earlier foetal death, and eight chronic medical conditions. Adjusted HR 0.30 (95% CI: 0.25 to 0.34). Unclear what	Data obtained from national population immunisation surveillance, birthing, and health reimbursement databases. Foetal death was defined earlier than the majority of other studies at greater than 12 weeks. Maternal risk factors described as per adjusted variables.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
					diagnosis following vaccination (R80 International Classification of Primary Care code)	covariates were included in the adjusted model.	
Irving et al. (2013)[79]	**Study design:** Case control study **Statistical methods:** Analysis involved a 3 level categorical variable for influenza vaccine exposure to the vaccine relative to the date of spontaneous abortion. The primary analysis included; 1) exposure 1–28 days before the reference date, 2) same season exposure more than 28 days before the reference date, and 3) unexposed as of the reference date. The secondary analysis;	**Setting:** USA health care sites linked by a data network **Participants:** Pregnant women who had experienced pregnancy loss during the study period, mean age 31.7 (SD±6.0) in group1 and 29.3 (SD±5.4) years in group 2. 16.5% of women had a chronic	Primary intervention of interest was trivalent influenza vaccination. **Group 1)** Pregnancy loss through the first 16 weeks gestation. (n=243) **Group 2)** Randomly matched women with no pregnancy loss through the	**Aim** Estimate the association between spontaneous abortion and influenza vaccine **Study period:** October 25, 2005 to February 4, 2006, and from October 22, 2006 to February 3, 2007. Periods chosen when the vaccine was available.	Association between spontaneous abortion and influenza vaccine receipt in the 28-day exposure window. Association between spontaneous abortion and influenza vaccine receipt. (preconception	Vaccinated 1-28 days prior to spontaneous abortion, adjusted OR 1.23 (95% CI: 0.53 to 2.89, p=0.63) Vaccinated more than 28 days prior to spontaneous abortion, adjusted OR 1.24 (95% CI: 0.54 to 2.86, p=0.61) Vaccinated while pregnant, adjusted OR 0.80 (95% CI: 0.36 to 1.78, p=0.58) Vaccinated pre conception, adjusted OR 2.34 (95% CI: 0.86 to 6.33, p=0.10)	Missing some potentially important confounding factors such as alcohol, recreational drug use, prescription drug use, race, and maternal weight. Maternal risk factors described included age, febrile illness during 1st trimester, smoking,

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	1) exposure after conception and before spontaneous abortion. 2) Same season exposure before conception and 3) Unexposed. In both analyses the unexposed category served as the referent group for both categories 1 and 2. Conditional logistic regression models adjusted for maternal age, parity, maternal diabetes and health care utilisation. Paired t-tests and McNemar tests were used.	condition reported. **Trimester vaccinated:** Either before conception or during the 1st trimester.	first 16 weeks gestation. (n=243)		and post conception) Post conception defined as last menstrual period plus 14 days.		diabetes, asthma, and hypertension. Diabetes and age were statistically different; both were included in the adjusted analysis.
Jackson et al. (2011)[56]	**Study design:** Randomised controlled trial **Statistical methods:** Data were presented as frequencies and percentages.	**Setting:** USA **Participants:** Pregnant women in good health (long list of exclusions). Mean age in both cohorts 31.7 range (20	**Group 1)** Two doses of 25μg HA influenza A (H1N1) 2009 split-virion, non- adjuvant vaccine, 21 days apart	**Aim:** Not stated, primarily immunologic study **Study period:** September 2009 to October 2009	Local reactions at first vaccination Systemic reactions at	Mild to moderate pain at injection site 25μg, 25%. 49μg, 35% Erythema < 50mm, 25μg, 8%. 49μg, 13% Swelling/induration, 25μg, 7%. 49μg, 2% Fever (not defined),	

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		to 39) and 31.2 range (18 to 39). **Trimester vaccinated:** 2nd or 3rd. Gestational age at vaccination group 1, 24.4 (SD±6.2), group 2, 22.6 (SD±6.0)	(n=60) **Group 2)** Two doses of 49µg HA influenza A (H1N1) split-virion, unadjuvanted vaccine, 21 days apart (n=60)		first vaccination Serious adverse events pregnant women Serious adverse events infants	25µg, 8%. 49 µg, 7% Malaise, 25 µg, 31%. 49µg, 40% Oral temperature >37.8, 25µg, 0%. 49µg, 2% Headache 25µg, 28%. 49µg, 30% 18 serious adverse events were reported for 15 pregnant women; all were considered to be unrelated to the vaccine. 24 serious events were reported for 20 infants; all were considered unrelated to the vaccine.	

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Kallen B, Olausson P. (2012)[91]	**Study design:** Retrospective cohort study **Statistical methods:** Mantel–Haenszel odds ratio and approximate 95% confidence intervals were estimated with Miettinen's method, adjusted for year of birth, maternal age, parity, smoking, BMI, preterm birth, low birth weight, and SGA<2 SD.	**Setting:** Population cohort from Sweden **Participants:** Pregnant women who delivered after 1st of October 2009 and another group who delivered prior to that date. 80.2% of vaccinated cohort were < 35 years of age, 77.5% of unvaccinated cohort were < 35 years of age. **Trimester vaccinated:** All trimesters, 3165 (17.0%) in the 1st trimester.	**Group 1)** Influenza A (H1N1) 2009, split virus vaccine AS03-adjuvant and thiomersal preservative. Pregnant women: (n=18612) Infants: (n=18 844) **Group 2)** Non-vaccination group Pregnant women: (n=136 914) Infants: (n=138 931) **Group 3)** Pre-vaccination group who	**Aim:** Describe a large study on pregnancy outcome after vaccination against (H1N1) during the 2009/10 pandemic. **Study period:** October 2009 to December 2010. Study period started at the start of the mass vaccination period. Not clear in regards to timing of peak influenza period.	Stillbirth (unclear of definition used) Preterm birth (< 37 weeks, singleton births only) Low birth weight (< 2500g, singleton births only) SGA (<2 SD from expected weight at gestational age, singleton births only)	Vaccinated versus non-vaccinated 0.77 (95% CI: 0.57 to 1.03), vaccinated vs. pre-vaccination group 0.81 OR (95% CI: 0.59 to 1.12) Vaccinated versus non-vaccinated OR 0.86 (95% CI: 0.77 to 0.96), vaccinated versus pre-vaccination group OR 0.91 (95% CI: 0.83 to 1.00) Vaccinated versus non-vaccinated OR 0.86 (95% CI: 0.77–0.96), vaccinated versus pre-vaccination group OR 0.91 (0.81 to 1.03) Vaccinated versus non-vaccinated OR 1.04 (95% CI: 0.92 to 1.17), vaccinated versus pre-vaccination group OR 1.08 (95%	Medical Birth Register database was used to access medical records of antenatal care, delivery, and neonatal condition of the newborn infant. Maternal risk factors; age, parity, smoking and BMI were the only described.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		Numbers in 2nd and 3rd not reported. Timing of vaccination data was missing in 18% of participants.	gave birth in the year 2009 before October Pregnant women: (n=83 298) Infants: (n=84 484)		Congenital malformations first trimester only (any congenital malformation in the Medical Birth Register. Common and less significant malformations were excluded) Women with known vaccination week compared with non-vaccinated women who were still pregnant in that week	CI: 0.95 to 1.23) Vaccinated versus non-vaccinated OR 1.01 (95% CI: 0.83 to 1.23), vaccinated versus pre-vaccination group OR 1.04 (95% CI: 0.85 to 1.28) Stillbirth OR 0.95 (95% CI: 0.69 to 1.22), Preterm birth among singletons OR 0.96 (95% CI: 0.88 to 1.05), Low birth weight in singletons OR 0.96 (95% CI: 0.87 to 1.07) SGA OR 1.05 (95% CI: 0.94 to 1.18).	
Lim SH et al. (2010)[92]	**Study design:** Single arm prospective survey	**Setting:** Obstetric clinics in Korea **Participants:**	**Group 1)** Influenza A (H1N1) 2009 split-virion,	**Aim:** Not stated **Study period:**	Local adverse events	Soreness (41.6%) and redness (8.4%) at the injection site.	Participants were asked to complete a daily log

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	Statistical methods: Data were presented as frequencies and percentages.	Healthy pregnant women. Mean age was 31.3 (SD±3.8) years **Trimester vaccinated:** All trimesters, 2 (1.1%) in the 1st trimester, 75 (40.1%) in the 2nd, and 110 (58.8%) in the third trimester. 3 not reported.	non-adjuvant vaccine. (n=190) Followed until birth (n=162)	December 2009 to January 2010.	Systemic adverse events	Fatigue (36.6%), myalgia (23.7%), dizziness (23.2%), headache (20%), fever (13.7%), chills (10%), respiratory symptoms including rhinorrhoea (14.2%), sore throat (11.1%), cough (8.4%), gastrointestinal symptoms including diarrhoea (5.8%), and abdominal pain (5.3%).	following vaccination. Attrition of 28 women in total. Other than age no maternal risk factors were described
Lin et al. (2012)[93]	**Study design:** Retrospective cohort **Statistical methods:** Data were presented as frequencies and percentages. Univariate analysis was also conducted using the Chi-square test for proportions.	**Setting:** Medical centres in Taiwan **Participants:** Pregnant women. Mean age for both cohorts was 32.4 (SD±4.0) and 32.8	**Group 1)** Influenza A (H1N1) 2009, non-adjuvant, split-virus vaccine. Pregnant women (n=198) Infants	**Aim** Observe the safety profile of a specific influenza A (H1N1) vaccine in pregnant women **Study period:** From October 2009 to	Adverse events within 1 week Adverse events duration of pregnancy	2.0% had adverse drug reactions including fever, cough, runny nose, nasal congestion, and skin itching. 8.6% of the vaccinated group and 20.2% of the unvaccinated group had at least one adverse event during	Maternal risk factors described included age, smoking, history of preterm birth, and socioeconomic status. There was no

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		(SD±3.9) years. Chronic disease history not reported. **Trimester vaccinated:** Any trimester, 10 (4.9%) in the 1st, 82 (41.4%) in the 2nd trimester, and 106 (53.3%) in the 3rd trimester.	(n=202) Group 2) Not vaccinated with influenza vaccine. Pregnant women (n=198) Infants (n=206)	February 2010	Adverse events in infants Premature birth < 37 weeks Premature birth < 35 weeks Stillbirth (unclear of definition) URTI in the first 8 weeks following birth Low birth weight < 2500g	their pregnancy. Conditions such as optic neuritis, cranial neuropathy, or Guillain-Barre syndrome were not reported. 72 (35.6%) vaccinated, 101 (49%) unvaccinated within 8 weeks after they were born. (p< 0.05) 12 (5.9%) vaccinated, 18 (8.7%) unvaccinated. 2 (0.9%) vaccinated, 7 (3.4%) unvaccinated (p=0.09) Nil in the vaccinated, cohort and 1 in the unvaccinated cohort. 6 (3.0%) vaccinated, 8 (3.9%) unvaccinated 16 (7.9%) vaccinated, 28 (13.6%) unvaccinated	statistical difference in either cohort.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Mackenzie et al. (2012)[94]	**Study design:** Prospective cohort study **Statistical method** Descriptive outcomes only considered for this review.	**Setting:** Scotland and UK general practices **Participants:** Sub population of pregnant women. Characteristics not reported. **Trimester vaccinated:** 19 (14.7%) in the 1st, 53 (45%) in the 2nd, and 30 (24%) in the 3rd trimester. 2 were not reported.	**Group 1)** Influenza A (H1N1) 2009 vaccine. Type not stated. (n=104) **Group 2)** Not vaccinated influenza vaccine. (n=13)	**Aim:** Establish the feasibility of rapidly monitoring the new swine flu vaccines in large patient numbers. To describe pregnancy outcomes in vaccinated and non- vaccinated women. **Study period:** 2 November 2009 and closed to new recruitment on 30 April 2010. The study period coincided with the vaccination program timing.	Serious adverse events for pregnant women. Serious adverse events for infant at birth. Spontaneous abortion (not defined)	No serious adverse events were reported. There were six potential congenital abnormalities cases reported involving hypospadias, Down's syndrome, hydrocephalus, umbilical hernia, cleft palate, and skin tag on finger. None were reported in the 13 unvaccinated women. There were reports of 4 spontaneous abortions in in 3 women.	Pregnant women comprised a small sub population of a broader study. Active surveillance occurred at monthly intervals. Pregnancies were followed up to delivery date or to the end of pregnancy. Outcomes of 29 births were lost to follow up Maternal risk factors not described

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Munoz et al. (2005)[95]	**Study design:** Retrospective cohort study **Statistical method** Data were presented as frequencies and percentages. Continuous values were analysed with t-test for comparison of the means. Nominal values were compared among the groups with chi-squared test or Fisher exact test.	**Setting:** Clinic in USA **Participants:** Healthy pregnant women who had uncomplicated singleton pregnancy. Mean age 30.7 years. A comparison group was selected by matching of maternal age at delivery, month of delivery, and type of insurance. Mean age 30.8 years. **Trimester vaccinated:** 2nd or 3rd. Numbers not	**Group 1)** Seasonal influenza vaccine. Antigenic make up of vaccines not reported. Infants and mothers: (n=225) **Group 2)** Not vaccinated with influenza vaccine. Infants and mothers: (n=826)	**Aim:** Evaluate the safety of influenza vaccine that is administered in the second or third trimester of gestation **Study period:** 5 influenza seasons from July 1, 1998, to June 30, 2003. The allocation of the study period appears to be the putative influenza season. Prevalent strains listed in paper and peak influenza periods. The match of the vaccines is unclear.	Serious adverse events (hospitalisation, medical visits within 42 days after vaccination) Premature birth Medically attended acute respiratory tract illness in pregnant women (from time of vaccination to delivery) Acute respiratory illness during the peak	9 women were hospitalised within 14 days of vaccination. 11 women were hospitalised for reasons that related to delivery. 2 were not related to delivery and 1 had influenza-like illness within 5 days of receipt of vaccine. 5.5% vaccinated 8% unvaccinated OR 0.67 (95% CI: 0.32 to 1.32, p=0.28) 51 (22.6%) vaccinated 156 (18.9%) unvaccinated (p=0.24). 26.8% vaccinated 30.2% unvaccinated	Electronic database search obtained ICD-9 codes on common diagnosis and complications that may occur prior to delivery. Healthy pregnant women and matched cohort, no maternal risk factors described.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		specified; the mean gestational age at vaccination was 26.1 weeks.			influenza season for infants < 6 months. Pneumonia for infants < 6 months. (Excluding month 1) Congenital malformation	1.6% vaccinated, 2% unvaccinated Nil in vaccinated, 15 (1.8%) unvaccinated.	
Nordin et al. (2013)[82]	**Study design:** Retrospective cohort study **Statistical methods:** Chi-square and Mann-Whitney U tests to compare baseline characteristics. Generalised estimating equation method to account for the matching effect, with a Poisson distribution and log link calculated maternal incident rate ratios.	**Setting:** 10 health care systems across the USA. **Participants:** Pregnant women with singleton pregnancies. Mean age 30.8 (SD±5.6) years. The vaccinated cohort were more likely to	**Group 1)** Trivalent seasonal influenza vaccine. Antigenic make up of vaccines not stated. (n=75906) **Group 2)** Pregnant women not vaccinated with influenza	**Aim:** Estimate the risks for medically attended events occurring within 42 days of receiving trivalent inactivated influenza vaccine and to evaluate specific risks of first-trimester vaccination.	0-3 day adverse events First 42 days after vaccination adverse events	Adverse event in the first 3 days after vaccination, adjusted incident rate ratio (IRR) 1.12, (95% CI: 0.81 to 1.55). Adverse event in days 1 to 42 post influenza vaccination, adjusted IRR 0.90 (95% CI: 0.68–1.19) New diagnosis of thrombocytopenia, adjusted IRR 0.90 (95% CI: 0.68 to 1.19).	Comparison of rates of medically attended adverse events in pregnant women from Vaccine Safety Datalink. An algorithm identified pregnant episodes and matched vaccinated and unvaccinated

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	Adjusted for pre-existing high-risk conditions, receipt of care in the first trimester, and hospitalisation before the vaccination or index date.	have a pre-existing condition (14.5% compared with 11.7%) **Trimester vaccinated:** 1 21553 (28.4%) 1st trimester, 33553 (44.2%) 2nd trimester, and 20800 (27.4%) 3rd trimester.	vaccine matched by age, site, and estimated pregnancy start date. (n=126246)	**Study period:** June 2002 to July 31, 2009		Acute neurologic event, adjusted IRR 0.92 (95% CI: 0.54 to 1.56). Among vaccinated women there were no cases of Guillian-Barré syndrome, optic neuritis, Bell's palsy, or transverse myelitis.	women who would be likely to be vaccinated in the same risk window.
Omer et al. (2011)[96]	**Study design:** Retrospective cohort study **Statistical method** The primary adjusted models identified covariates that produce adjusted ORs of 1 during the pre-influenza period. Covariates assessed included; gestational age for first antenatal visit, maternal diabetes	**Setting:** multistate surveillance USA **Participants:** Pregnant women, 82.3% of vaccinated cohort was under 35 compared to 85.4% of unvaccinated	**Group 1)** The receipt of inactivated seasonal influenza vaccine. Type not reported. (n=578) **Group 2)** No seasonal influenza vaccine	**Aim:** Evaluate whether there is an association between receipt of inactivated influenza vaccine during pregnancy and birth outcomes. **Study period:** A pre and peak influenza period	SGA (< 10th percentile for gestational age) Premature (< 37 weeks)	Putative influenza season OR 0.74 (95% CI: 0.47 to 1.15) Period of widespread influenza activity OR 0.31 (95% CI: 0.13 to 0.75) Putative influenza season adjusted OR 0.60 (95% CI: 0.38 to 0.94, p=0.02) Period of widespread	Birth related data were extracted from Pregnancy Risk Assessment Monitoring System database. Trimester of vaccination not established. Maternal risk

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	(gestational and/or non-gestational), multivitamin use in pregnancy, history of alcohol use during pregnancy, education less than 12th grade, and mother married. Logistic regression was used to evaluate the association of maternal influenza vaccine with prematurity and SGA infants.	cohort. **Trimester vaccinated:** Not reported, unable to be obtained from database.	(n=3590)	was identified using laboratory and case reports during the 2004 - 2005 and 2005 - 2006 influenza season. Circulating strains or match of vaccine not reported.		influenza activity OR 0.28 (95% CI: 0.11 to 0.74, p=0.01)	factors investigated included those listed as covariates plus smoking, race and hypertension were also used in a secondary adjusted model.
Omon et al. (2011)[97]	**Study design:** Prospective single arm study **Statistical methods:** Data were presented as frequencies and percentages.	**Setting:** France national monitoring survey **Participants:** Pregnant women, mean age 31 (SD±4) years. **Trimester vaccinated:** All trimesters, 29 (4%) < 15 weeks, 308	**Group 1)** Influenza A (H1N1) 2009 vaccine. Non-adjuvanted, split-virion vaccine. (n=651) Birthing outcomes (n=580)	**Aim:** Describe pregnancy outcomes among women vaccinated with non-adjuvanted influenza vaccines in South Western France **Study period:** October 2009 to February 2010	Adverse events Adverse events during pregnancy Hospitalised during pregnancy	Fever occurred in 11 (1.9%) women. Also reported were asthenia, headaches, and pain at the site of injection. Influenza-like symptoms occurred in 11 (1.9%) women. 141 (25%) women. 56 (9.6%) mainly related to hypertension or preterm labour.	Three successive standardised questionnaires were used. The first of these was filled in when the vaccine was administered. The 2nd and 3rd conducted via a phone interview. Comparisons

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		47% < 28 weeks, and 313 (48%) > 28 weeks.			Premature birth < 37 weeks. Foetal death (Definition unclear) Major congenital abnormalities (EUROCAT definitions)	42 (7.2%) of women vaccinated gave birth prematurely. One foetal death was reported and no stillbirths. 19 (3.1%) reported	of incidence were made with external surveys. Attrition of 82 women. Maternal risk factors described for maternal age, premature birth, diabetes, hypertension, folic acid supplementation, alcohol, and tobacco consumption.
Opperman n et al. (2012)[77]	**Study design:** Prospective cohort study **Statistical methods:** Cox proportional hazards model were used with vaccination a time dependent covariate for spontaneous abortion.	**Setting:** Germany **Participants:** Pregnant women, median age 33 in vaccinated cohort and 32 in control group.	**Group 1)** Non-adjuvanted split-virion influenza A (H1N1) 2009 vaccine (n=216). AS03-adjuvanted	**Aim:** Estimate the risk for spontaneous abortions and major birth defects. Preeclampsia, gestational age at birth, and birth weight were	Spontaneous abortion (definition unclear) Stillbirth (definition unclear) Preeclampsia All	Adjusted HR 0.89 (95% CI: 0.36 to 2.19) adjusted for study entry and vaccination time Nil Adjusted OR 1.15 (95% CI: 0.54 to 2.46). Adjusted OR 0.92	Data were collected via three structured questionnaires at first contact, six weeks, and eight weeks after the estimated date

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	Logistic regression was performed for major malformations and pre-eclampsia. A propensity score was calculated and the logit of the propensity score was included as a covariate in the logistic regression. Included as covariates in the propensity score were BMI, age, alcohol consumption, smoking habits, drug consumption, number of previous pregnancies, number of previous foetal losses, number of previous children with malformation, other diseases, and family medical history.	Random samples of all available pregnant women controls with an estimated date of birth in the range of the study cohort were selected. **Trimester vaccinated:** All trimesters, 20 (6.2%) preconception, 55 (17%) in the 1st, 144 (44.6%) in the 2nd, 104 (32.2%) in the 3rd trimester.	vaccine influenza A (H1N1) 2009 vaccine (n=90). MF59 adjuvanted subunit influenza A (H1N1) vaccine (n=2). For 15 women the vaccine type could not be ascertained. Total (n=323) **Group 2)** Pregnant women who were not vaccinated with influenza A (H1N1) 2009 vaccine (n=1329)	evaluated as secondary endpoints **Study period:** April 2009 to June 2010	malformations (EUROCAT definitions) Major malformations All malformations following 1st trimester and vaccination prior to conception. Premature birth < 37 weeks	(95% CI: 0.58 to 1.46) Adjusted OR 1.11 (95% CI: 0.51 to 1.46). Crude OR 0.99 (95% CI: 0.43 to 2.00). 29 (9.09%) vaccinated 122 (10.25%) unvaccinated	of birth for vaccinated cohort and control group. The term 'spontaneous abortion' and 'stillbirth' are not described in the paper. This study combines pre-conception vaccination (4 weeks prior to conception). It is unable to be separated from the overall data. 312 women lost to follow up. Maternal risk factors investigated as per covariate list.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Pasternak et al. (2012)[98]	**Study design:** Retrospective cohort study **Statistical methods:** Kaplan-Meier method to generate survival curves according to vaccination status. Cox proportional hazards regression with gestational age in days as the underlying time scale. Logistic regression was used to estimate each woman's probability to be vaccinated conditional on covariates. Women were excluded with a non-overlapping probability of being vaccinated at the extreme end of score distribution. Propensity models included the following variables: Maternal age, county of residence, degree of urbanisation,	**Setting:** Nationwide registry in Denmark **Participants:** All singleton pregnancies (live births, stillbirths and pregnancies with an abortive outcome) in Denmark. Pregnant women mean age 30 (SD±5.2) in the vaccinated cohort and 30.9 (SD±4.7) in the unvaccinated. Overall percentage of women with a chronic disease unable to be	**Group 1)** Influenza A (H1N1) 2009 inactivated AS03-adjuvanted split-virion vaccine. Foetal death analysis. (n=7062) Stillbirth analysis. (n=7014) Spontaneous abortion analysis. (n=2736) **Group 2)** Not vaccinated with influenza A (H1N1) vaccine. Foetal death analysis. (n=47524) Stillbirth	**Aim:** To investigate whether an adjuvanted pandemic A (H1N1) 2009 influenza vaccine in pregnancy was associated with an increased risk of foetal death. **Study period:** November 2009 to 30 September 2010. Coincided with the start of the vaccination campaign.	Foetal death (spontaneous abortion and stillbirth combined) Foetal death in pregnant women in the 2 weeks following vaccination Spontaneous abortion (week 7 to week 22) Spontaneous abortion in the 2 weeks following vaccination Stillbirth (delivery of a dead foetus after 22 weeks)	Adjusted HR 0.79 (95% CI: 0.53 to 1.16) Adjusted HR 0.48 (95% CI: 0.22 to 1.10) Adjusted HR 1.11 (95% CI: 0.71 to 1.73) Adjusted HR 0.47 (95% CI: 0.19 to 1.13). Adjusted HR 0.44 (95% CI: 0.20 to 0.94	Information was obtained from the medical birth register that contains detailed records of all births in Denmark. Pregnancies with abortive outcomes were identified using ICD-10 coding. Maternal risk factors investigated as per covariate list.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	country of birth, parity, history of foetal death in siblings, selected comorbidities, number of hospital admissions and outpatient hospital contacts within three years preceding pregnancy, selected drugs and number of drugs used within six months before pregnancy.	calculated. **Trimester vaccinated:** Any trimester, 2736 (7.7%) in the 1st trimester. 2nd and 3rd trimester numbers not reported for individual trimesters.	analysis. (n=43663) Spontaneous abortion analysis. (n=32627)				

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Pasternak et al. (2012)[46]	**Study design:** Retrospective cohort study **Statistical methods:** Analysis was separated into vaccination in the 1st trimester and vaccination in the 2nd or 3rd. Those vaccinated in the 1st trimester were excluded from the analysis of 2nd and 3rd trimester vaccinations. A propensity score for each participant was estimated using logistic regression as the predicted possibility of vaccination conditional on potential confounders. Following estimation of propensity scores both cohorts were matched 1:1 and participants with no match were excluded. Logistic regression was used to estimate	**Setting:** Nationwide registry in Denmark **Participants:** Pregnant women with live born singleton infants. Excluded known causes and congenital viral infections possibly associated with birth defects and unspecified congenital viral disease. Pregnant women mean age 30.7 (SD±5.2) in the vaccinated cohort and 30.1 (SD±5.0) in the	**Group 1)** Influenza A (H1N1) 2009 inactivated AS03-adjuvanted split-virion vaccine. 1st trimester (n=345), propensity score matched analysis (n=330) 2nd and 3rd trimester (n=6644), propensity score matched analysis (n=6642) **Group 2)** Not vaccinated with influenza vaccine 1st trimester (n=22917), propensity	**Aim:** To investigate whether exposure to unadjuvanted influenza A (H1N1) 2009 vaccine during pregnancy was associated with increased risk of adverse foetal outcomes. **Study period:** November 2, 2009 to September 30, 2010. Coincided with the start and end of the vaccination campaign.	Major birth defects following vaccination in 1st trimester (defined using EuoroCAT criteria) Preterm birth < 37 weeks vaccinated during their 1st trimester) Preterm birth < 37 weeks vaccinated during their 2nd and 3rd trimester) Very preterm birth (<32 weeks, 2nd and 3rd trimester vaccination) Low birth weight (<2500g, 1st	POR, 1.21 (95% CI: 0.60 to 2.45). POR, 1.32 (95% CI: 0.76 to 2.31) POR, 1.00 (95% CI: 0.84 to 1.17). POR 0.97 (95% CI: 0.63 to 1.53) POR 0.83 (95% CI: 0.41 to 1.67).	Information was obtained from the medical birth register that contains detailed records of all births in Denmark. Vaccination status was obtained from a national (H1N1) vaccination database. The population eligible for the vaccine during the first trimester was women with comorbidities. Maternal risk factors described as per covariate

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	prevalence odds ratios. Potential confounders in propensity matched scores included maternal age, place of birth, degree of urbanisation, parity, smoking, pre-pregnancy BMI, history of birth defects, preterm birth, spontaneous abortion, SGA, maternal comorbidities, use of drugs, health care utilisation, number of hospital admissions and hospital outpatient visits in the last 3 years, and number of drugs used in the last 6 months.	unvaccinated. **Trimester vaccinated:** All trimesters, 345 in the first trimester. 2nd and 3rd trimester numbers not reported for individual trimesters.	score matched analysis (n=330) 2nd and 3rd trimester (n=46443), propensity score matched analysis (n=6642) Small variations in preterm birth cohort sizes.		trimester vaccination) Low birth weight (<2500g, 2nd and 3rd trimester vaccination) Very low birth weight (<1500g, vaccinated in 2nd or 3rd trimester) SGA (<10th percentile for gestational age, 1st trimester vaccination) SGA (<10th percentile for gestational age, 2nd and 3rd trimester vaccination)	POR 1.14 (95% CI: 0.94 to 1.38) POR 1.11 (95% CI: 0.67 to 1.83) POR 0.79 (95% CI: 0.46 to 1.37) POR 0.97 (95% CI: 0.87 to 1.09)	list.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Poehling et al. (2011)[99]	**Study design:** Case control study **Statistical methods:** 3 Multivariate logistic regression models were used. The most comprehensive model included age, sex, race/ethnicity, site, study year, tertile of the influenza season, smoke exposure at home, number of siblings, day care attendance, insurance status, and whether the infant was ever breast-fed.	**Setting:** 3 county hospitals in the USA **Participants:** Inpatients <6 months of age with respiratory illness and/or fever. 44.0% female, 56% male. 11.8% were premature infants and 7.9% had a high-risk condition. **Trimester vaccinated:** Unclear	Primary intervention of interest was vaccination status during pregnancy. **Group 1)** Hospitalised influenza positive infants. (n=151) **Group 2)** Hospitalised infants with respiratory illness without laboratory-confirmed influenza (n=1359)	**Aim:** To determine whether maternal vaccination during pregnancy was associated with a reduced risk of laboratory-confirmed influenza hospitalisations in infants < 6 months old. **Study period:** 2002 to 2009 November to April, prior to influenza A (H1N1) 2009. Unclear on the match of the vaccines with circulating strain. The periods commenced from the first to	Hospital presentations for children under 6 months (PCR/viral culture)	12% of mothers of influenza-positive infants and 20% of mothers of influenza-negative infants were vaccinated. Adjusted OR of 0.52 (95% CI: 0.30 to 0.91) adjusted as described in the statistical method.	Infants were identified through hospital surveillance with fever and/or acute respiratory symptoms.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
				last influenza-positive nasal/throat swab among study infants.			
Richards et al. (2013)[80]	**Study design:** Retrospective cohort study **Statistical methods:** Logistic regression to evaluate the association between primary outcomes was performed. Primary analyses adjusted for: maternal age, asthma, gestational diabetes, cardiovascular disease, hypertension during pregnancy, multiple birth, any pregnancy/birth complication, any antiviral use during pregnancy, and site. Propensity score regression and matching methods were used in	**Setting:** Health region in USA **Participants:** Pregnant women with live births. Mean age 31.2 (SD±5.6), 4% with asthma, 15.7% diabetes, and 6% cardiovascular disease. **Trimester vaccinated:** Unknown trimester of vaccination. Data not collected.	**Group 1)** Monovalent influenza A (H1N1) 2009 vaccine (n=1125). 759 of this cohort also received the seasonal trivalent inactivated vaccine. Vaccine type not reported. **Group 2)** Not vaccinated with influenza vaccine (n=1581)	**Aim:** Not stated **Study period:** 26 April 2009 to 17 April 2010. Season defined by first positive laboratory test up to the first week when the percentage of influenza A (H1N1) was <5%, (including births before and after the start of availability of 2009 (H1N1) influenza A vaccine).	Vaccine effectiveness (Case defined as having a reverse transcription polymerase chain reaction test positive for influenza, or having a medical visit during pregnancy with influenza-related ICD-9 diagnosis code during the period of 2009 influenza A (H1N1) virus circulation.)	61.5% (95% CI: 15.5% to 82.5%).	All births in the participating health sites were identified via electronic medical records and then restricted to mothers who had the opportunity for 3rd trimester exposure to 2009 influenza A (H1N1) virus. Potential for selection bias with participants included prior to the vaccine becoming

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	sensitivity analysis. Vaccine effectiveness was calculated as 1 minus adjusted odds ratio.				Preterm birth < 37 weeks. SGA < 10th percentile Low birth weight (<2500g)	Adjusted OR, 0.63 (95% CI: 0.47 to 0.84) Adjusted OR 1.26 (95% CI: 0.94 to 1.69) Adjusted OR 0.79 (95% CI: 0.56 to 1.10).	available. Maternal risk factors described as per covariate list. Missing data for race, smoking, and alcohol use and a smaller sensitivity analysis was performed on these potential confounders.
Rubinstein et al. (2013)[83]	**Study design:** Cross sectional study **Statistical methods:** Multiple logistic regression analysis was performed. Retained variables included antenatal visits, level of education, maternal age, income, parity, smoking, and history of pregnancy induced hypertension. A Propensity score	**Setting:** 49 public hospitals in Argentina **Participants:** Pregnant women with a live born or stillborn infant of at least 22 weeks gestation or weighing 500g	**Group 1)** Monovalent Influenza A (H1N1) 2009 vaccine MF-59-adjuvanted subunit vaccine. (n=7293) **Group 2)** Not vaccinated influenza	**Aim:** To assess the risk of adverse perinatal events of vaccination of pregnant women with an MF59 adjuvanted vaccine. **Study period:** September 2010 to May 2011, no mention of	Preterm delivery (<37 weeks) Low birth weight (<2500g) Perinatal mortality (early neonatal mortality plus foetal mortality)	Adjusted OR 0.79 (95% CI: 0.69 to 0.90) Adjusted OR 0.74 (95% CI: 0.65 to 0.83). Adjusted OR 0.68 (95% CI: 0.42 to 1.06).	A consecutive sample of all women who delivered in the participating hospitals were eligible to participate. Information was extracted from the medical records and

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	analysis was used for an outcome not extracted for this review.	or more at birth. 88.5% of vaccinated cohort < 35 years, 86.9% in the unvaccinated cohort. Vaccinated cohort had more antenatal clinic visits and earlier first visit and was a statistically significant difference. **Trimester vaccinated:** Any, 2874 (39.4%) in the 1st trimester, 3545 (48.6%) in the 2nd, 736 (10.1%) in the 3rd, 138 (1.9%) not known.	vaccine (n=23195)	circulating strain or match of vaccine	Foetal death (over 22 weeks) Neonatal death (7 days post-partum) Congenital malformation Neonatal intensive care admission Very low birth weight (1500g) Non-immune jaundice Pre-eclampsia	25 (0.3%) vaccinated, 111 (0.5%) unvaccinated (p=0.08) 29 (0.4%) vaccinated, 146 (0.6%) unvaccinated (p=<0.01) 35 (0.5%) vaccinated, 137 (0.6%) unvaccinated 433 (5.9%) vaccinated, 1523 (6.6%) unvaccinated 44 (0.6%) vaccinated, 306 (1.3%) unvaccinated (p=<0.01) 273 (3.7%) vaccinated, 706 (3.0%) unvaccinated (p=0.012) 124 (1.7%) vaccinated, 384 (1.7%) unvaccinated	survey. Possibility of selection bias due to severely unwell women not being enrolled as they would have been unable to provide consent. Maternal risk factors described as per covariate list plus maternal weight.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Sammon et al. (2012)[100]	**Study design:** Retrospective cohort study **Statistical methods:** Discrete survival analysis, weekly intervals were used to define exposure and event occurrence and separate hazard ratios were estimated for weeks 9–12, weeks 13–24, and weeks 25–42. Two models were used; 1) Assess if vaccination has an adverse association with foetal death ('Toxicity model'). 2) Assess if vaccination has a protective effect against foetal death ('Immunity model'). Potential confounders investigated in the analysis include: maternal age, history of spontaneous loss, diabetes, pre-pregnancy	**Setting:** UK General Practices **Participants:** Pregnant women with at least 6 months of data available before their last menstrual period date. Mean age 29.9 for those who delivered an infant. 5.9% were in another clinical risk group for influenza and 24.6% smoked. **Trimester vaccinated:** All trimesters. Trimester vaccination reported as vaccinated	**Group 1)** Influenza A (H1N1) 2009 vaccine. (n=9445) **Group 2)** Not vaccinated influenza vaccine. (n=26993)	**Aim:** Investigate whether the hazard of foetal death is altered in pregnancies vaccinated against influenza A (H1N1) 2009 **Study period:** Pregnancies ending after the start of the vaccination campaign on 21 October 2009 and conception prior to 1st January 2010.	Foetal death (defined as a loss at any time between the 9th week labour/delivery)	Foetal death in weeks 9–12, immunity model HR 0.74 (95% CI: 0.62 to 0.88), toxicity model 0.56 (95% CI: 0.43 to 0.73). Foetal death in weeks 13–24, immunity model 0.59 (95% CI: 0.45 to 0.77), toxicity model 0.45 (95% CI: 0.28 to 0.73). Foetal death in weeks 25–43, immunity model 0.70 (95% CI: 0.47 to 1.03), toxicity model 1.56 (95% CI: 0.73 to 3.34). The protective association with influenza A (H1N1) 2009 vaccine was similar during periods of high influenza circulation and little or no influenza circulation. No variables were	Data was obtained from the UK General Practice Research Database that contains primary care records for 8.4% of the UK population. an algorithm was used to identify pregnancies and estimate start and end dates. Maternal risk factors described as per covariate list.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	smoking status, pre-pregnancy alcohol use, pre-pregnancy body mass index, the number of consultations in the 6 months before the last menstrual period, and being in an influenza risk group.	weeks included in the models. Vaccinated weeks were 2.9% 1st trimester, 24.4% in the 2nd, and 72.7% in the 3rd.				observed to confound the association between vaccination and foetal death and the authors thought that residual confounding was present.	
Sheffield et al. (2012)[101]	**Study design:** Retrospective cohort study **Statistical method** Student's t-test, chi-square test, and logistic regression. In a small number of outcomes the authors adjusted for race and diabetes.	**Setting:** Hospital system in the USA **Participants:** Pregnant women. The vaccinated cohort had 89% of women who were under 35, the unvaccinated cohort was 91%. The vaccinated cohort had a history of	**Group 1)** Seasonal trivalent influenza vaccine Mothers (n=8690) Infants (n=8864) **Group 2)** Not vaccinated with influenza vaccine Mother (n=76153) Infants (n=76919)	**Aim** Estimate the effect of first-trimester influenza vaccination on foetal and neonatal outcomes. **Study period:** Five influenza seasons between October 2003 and March 2008. Unclear on the severity of the circulating	Premature birth (<37 weeks), Very premature birth (<32 weeks) SGA (< 10th percentile) Hyperbilirubinemia Stillbirth (Foetal death with weight ≥	460 (5%) vaccinated, 4 612 (6%) unvaccinated, (p=0.004) 65 (0.7%) vaccinated, 962 (1.3%) unvaccinated, (p<0.001) 944 (11%) vaccinated, 8,183 (11%) unvaccinated, (p=0.9) 305 (3%) vaccinated, 2694 (4%) unvaccinated, (p=0.7) 30 (0.3%) vaccinated 436 (0.6%) unvaccinated,	Data was obtained through a hospital system computerised database and discharge records. Possible selection bias with a higher percentage of women in the vaccinated cohort followed up through clinics.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		obstetric complication 72% compared to 48%, and diabetes 12% to 6%. Both were statistically significantly different. **Trimester vaccinated:** All trimesters, 439 (5%) in the 1st trimester, 8,251 in the 2nd and 3rd trimesters.		strains or the match of the vaccine.	500g) Major malformations	(p=0.006) 136 (2%) vaccinated 1163 (2%), (p=0.9) 1st trimester unadjusted OR 0.67 (95% CI: 0.36 to 1.26). 2nd and 3rd trimester, unadjusted OR 1.01 (95% CI: 0.85 to 1.21).	Limited adjustment for potential confounding variables. Some statistically significant differences between cohorts. Maternal risk factors described for race, hypertension, diabetes, and BMI.
Steinhoff et al. (2012)[102]	**Study design:** Secondary analysis of randomised controlled trial **Statistical methods:** Calculated ORs for outcomes adjusted for selected characteristics using multiple logistic and linear regression.	Refer to Zaman et al. (2008**).**[59]	Refer to Zaman et al. (2008**).**[59]	**Study period:** August 2004 through to December 2005. Two periods were defined, with one when limited virus was thought to be circulating and	SGA < 10th percentile Low birth weight <2500g	Overall study period, unadjusted OR 0.63, (95% CI: 0.4 to 1.0). During peak influenza circulation adjusted 0.44 (95% CI: 0.19 to 0.99, p=0.05) Overall study period unadjusted OR 0.53 (95% CI: 0.2–1.4,	Secondary analysis of the data obtained in the study by Zaman et al. (2008)[59]

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
	Adjusted for gestational age at immunisation and interval from immunisation to delivery. Proportions assessed using chi-square test and Fisher exact tests.			one when local surveillance shows a period of increased virus circulation.	Premature birth <37 weeks Stillbirth (unclear definition)	p=0.2) During peak influenza period adjusted OR 0.17 (95% CI: 0.02 to 1.63, p=0.1) Overall study period unadjusted OR 0.72 (95% CI: 0.3 to 1.7, p=0.4) During peak influenza period adjusted OR 0.32 (95% CI: 0.05 to 2.29). 3 stillbirths in influenza vaccinated cohort and no stillbirths in the pneumococcal vaccinated cohort.	
Sumaya CV, Gibbs RS, (1979)[57]	**Study design:** Prospective cohort study **Statistical methods:** Descriptive outcomes only.	**Setting:** Hospital in the USA **Participants:** Pregnant females, 3 women were over 35 years of age. Other	**Group 1)** Monovalent inactivated whole influenza A/NJ/76 (H1N1) vaccine.	**Aim** Not stated. Primarily to assess the safety and immunogenicity of inactivated influenza virus vaccines	SGA (<2 SD from the mean weight) Congenital malformation	9 (16%) 3 had detectable congenital defects; inguinal hernia, phalangeal tag, and clubfeet.	Surveillance was performed however the method is not explained. Group allocation is also not described.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		characteristics not described. Women were matched for age, race, and parity with unimmunised mothers. **Trimester vaccinated:** 11 vaccinated in 2nd and 45 during the 3rd trimester	Single dose (n=56) **Group 2)** Unvaccinated against influenza (n=56)	administered during pregnancy. **Study period:** 1976-1977, unclear of peak influenza circulation timing.			Maternal risk factors other than age were not described.
Tavares F, et al. (2011)[103]	**Study design:** Single arm prospective observational study **Statistical method:** Data were presented as frequencies and percentages.	**Setting:** GP practices in England **Participants:** Pregnant women. Mean age was 30.9 and 17.6% had a pre-existing medical condition. **Trimester vaccinated:**	**Group 1)** Influenza A (H1N1) 2009 split-virion, ASO3 adjuvant vaccine. Single dose. (n=267)	**Aim:** Not stated **Study period:** The start of mass vaccination 31 October 2009 to 12 December 2009	Preterm delivery (<37 weeks) Very pre-term delivery (<32 weeks) Low birth weight (<2500g) Very low birth weight (<1500g)	14 (5.4%) 3 (1.1%) 21 (8.1%) 4 (1.5%)	Active and passive surveillance was performed. Comparisons were made to the published background rates for the general population. Attrition of 2 women.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
		Any, 15.7% were vaccinated during the 1st trimester.			Congenital anomaly vaccination during first trimester	Nil	Maternal risk factors described included age and pre-existing medical conditions.
					Congenital anomaly vaccination during any trimester	5 (1.9%)	
					Spontaneous abortion (foetal death < 24 weeks)	4 (3.3%)	
					Stillbirth (foetal death ≥ 24 weeks)	Nil	
					Medically attended adverse event within the 31 day post-vaccination	59 (22.1%). Most common were respiratory tract infections (3.8%) and urinary tract infections (3%).	
					Serious adverse event	34 (12.7%). These were mostly associated with an adverse	

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
					during the 6-month follow-up period	pregnancy outcome. No adverse events of special interest reported.	
Tsatsaris et al. (2011)[58]	**Study design:** Single arm prospective study **Statistical methods:** Data were presented as frequencies and percentages.	**Setting:** Perinatal centres in France **Participants:** Healthy women aged 18 to 45 years were eligible if they were pregnant and between 22nd to 32nd weeks of gestation. Median age 32.0 (30.1 to 36.4) **Trimester vaccinated:** 2nd and 3rd trimester only	**Group 1)** Influenza A (H1N1) 2009 split-virion, non-adjuvant vaccine Mothers: (n=107) Infants: (n=116)	**Aim** Evaluate the immunogenicity and transplacental antibody transfer of 2009 pandemic influenza A (H1N1) vaccine administered during pregnancy. **Study period:** 3 November to 4 December 2009	Serious adverse events Adverse events in pregnant women	Serious adverse events were reported for 13 women. An independent committee considered none related to the vaccine. No adverse events of special interest were reported. Local adverse events were pain 20 (19%), induration 3 (3%), and erythema 2 (2%). Systemic reactions; asthenia 24 (22%), headache 10 (9%), myalgia 3 (3%), arthralgia 2 (2%), hyperhidrosis 2 (2%), pyrexia 1 (1%), and chills 2 (2%).	Phase 2 clinical trial. Primarily an immunological study. Local and general reactions were collected during the 30 minutes following vaccination. Pregnant women then kept a diary of any adverse events. Pregnant women filled out questionnaires

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
					Influenza-like episodes in infants	28 infants had fever associated with another respiratory symptom during the study period.	at 1 and 6 months after birth about infant.
Yeager et al. (1999)[104]	**Study design:** Prospective single arm study **Statistical method** Data were presented as frequencies, and percentages were only considered for this review.	**Setting:** Community and obstetric clinics in the USA. **Participants:** Pregnant women. Mean age 25.8 (SD±5.8). No chronic conditions described. **Trimester vaccinated:** Unclear, mean gestational age at vaccination 26.0 (SD±8.9).	**Group 1)** Seasonal trivalent influenza vaccination, split-virion. Antigenic make-up not reported. (n=319)	**Aim** Determine the acceptance rate and incidence of adverse reactions to the influenza vaccine. **Study period:** November 1997 to March 1998	Adverse events for pregnant women	17 (5.3%) reported adverse reactions. All reactions were described as mild and consisted mostly of influenza-like symptoms (4.4%) and soreness at the injection site (0.9%). No other adverse events were noted, including premature birth.	

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
Zaman et al. (2008)[59]	**Study design:** Randomised Controlled trial. **Statistical method:** Proportions were assessed using the chi-square test and Fisher's exact test. Intention-to treat analysis was performed. Incidence rate ratios (IRRs) were calculated using Poisson regression models. Estimates of clinical effectiveness were calculated with the formula (1 – IRR) X 100.	**Setting:** Bangladesh **Participants:** Healthy pregnant women in their third trimester. Mean age of the control group was 25.1 years, with a range of (18.0 to 36.0) in the vaccinated group and 24.9 years, range (18.0 to 36.0) in the control group **Trimester vaccinated:** 3rd trimester only.	**Group 1)** Trivalent seasonal influenza A/New Caledonia/20/99 (H1N1) A/Fujian/411/2002 (H3N2), and B/Hong Kong/330/2001 Single dose Mothers (n=172) Infants (n=169) **Group 2)** Pneumococcal vaccine Mothers (n=168) Infants (n=167)	**Aim:** The primary goal was to assess the immunogenicity of pneumococcal vaccine in mothers and infants, influenza vaccine was chosen as the comparator. **Study period:** August 2004 through December 2005 Unclear how original study period was chosen.	Effectiveness in infants of vaccinated mothers at preventing: Positive influenza test. Clinic visit. Respiratory illness with any fever. Respiratory illness with temperature > 38 C. Effectiveness in mothers at preventing: Respiratory illness with any fever. Respiratory illness with temperature >	62.8% (95% CI: 5 to 85). 42.0% (95% CI: 18.2 to 58.8) 28.9% (95% CI: 6.9 to 45.7) 28.1% (95% CI: -4.6 to 50.6) 35.8% (95% CI: 3.7 to 57.2) 43.1% (95% CI: -9.0 to 70.3)	Double blind and randomised using computer sequencing. Mothers and families were unaware of study group. Mothers were asked to record axillary temperatures of their infants. Families were asked to bring infants who were ill to the study clinic for evaluation, influenza-antigen testing and treatment.

Study	Methods	Setting and Participants	Interventions and cohorts	Aim/study period	Outcomes of interest	Results	Notes
					38 C. Clinic visit. Minor local and systemic reactions. Local pain Fever within 72 hours	24.9 (95% CI: -43.9 to 60.8) 13 (7.6%) influenza, 20 (12.0%) pneumococcal (p=0.17) 7 (4.1%) influenza, 19 (11.4%) pneumococcal (p=0.01) 23 (13.4%) influenza, 21 (12.6%) pneumococcal (p=0.81)	

Appendix VI. Critical appraisal

Randomised Control Trial / Pseudo-randomised Trial

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
Englund et al. (1993)[55]	Y	U	U	Y	U	U	Y	Y	Y	Y
Jackson et al. (2011)[56]	Y	Y	Y	N	N	Y	U	Y	Y	Y
Zaman et al. (2008)[59]	U	Y	Y	Y	Y	Y	Y	Y	Y	Y
Steinhoff et al. (2012)[102]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Y
%	66.67	66.67	66.67	66.67	33.33	66.67	66.67	100.00	100.00	100.00

Comparable Cohort / Case Control Studies

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9
Benowitz et al. (2010)[84]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Cristiani et al. (2011)[54]	Y	Y	U	N	Y	Y	U	Y	Y
Deinard AS, Ogburn P. (1981)[78]	Y	Y	U	U	Y	Y	N	Y	Y
Fell et al. (2012)[85]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Hulka JF (1964)[89]	Y	Y	N	N	Y	Y	N	U	Y
Lin et al. (2012)[93]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Heikkinen et al. (2012)[87]	Y	Y	Y	Y	Y	Y	N	Y	Y
Sammon et al. (2012)[100]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Horiya et al. (2011)[88]	Y	Y	Y	N	U	U	U	Y	Y
Kallen B, Olausson P. (2012)[91]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Mackenzie et al. (2012)[94]	Y	Y	N	N	Y	Y	N	Y	Y
Omer et al. (2011)[96]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Oppermann et al. (2012)[77]	Y	Y	Y	Y	Y	Y	N	Y	Y
Pasternak et al. (2012)[46]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Pasternak et al. (2012)[98]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Sheffield et al. (2012)[101]	Y	Y	Y	N	Y	Y	N/A	Y	Y
Sumaya C, Gibbs R. (1979)[57]	Y	Y	Y	N	U	Y	U	Y	Y
Irving et al. (2013)[79]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Håberg et al. (2013)[18]	Y	Y	Y	Y	Y	Y	N/A	Y	Y

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9
Eick et al. (2011)[37]	U	Y	Y	Y	Y	Y	U	Y	Y
Munoz et al. (2005)[95]	U	Y	Y	U	Y	Y	N/A	Y	Y
Black et al. (2004)[7]	U	Y	Y	N	Y	U	N/A	Y	Y
Poehling et al. (2011)[99]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Rubinstein et al. (2013)[83]	Y	Y	Y	Y	Y	Y	N/A	Y	Y
Conlin et al. (2013)[81]	U	Y	Y	U	Y	Y	N/A	Y	Y
Nordin et al. (2013)[82]	Y	Y	Y	U	Y	Y	N/A	Y	Y
Richards et al. (2013)[80]	Y	Y	Y	Y	Y	Y	N/A	Y	U
%	85.19	100.00	85.19	59.26	92.50	92.50	00.00	96.30	96.30

Descriptive studies

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9
Candela et al. (2012)[53]	N	Y	N	Y	N/A	Y	N	Y	Y
Lim et al. (2010)[92]	N	Y	U	Y	N/A	Y	N	Y	Y
Omon et al. (2011)[97]	N	Y	Y	Y	N/A	Y	N	Y	Y
Tavares et al. (2011)[103]	N	Y	Y	Y	N/A	Y	U	Y	Y
Tsatsaris et al. (2011)[58]	N	Y	Y	Y	N/A	Y	U	Y	Y
Yeager et al. (1999)[104]	N	Y	N	Y	N/A	Y	U	Y	Y
Harmark et al. (2011)[86]	N	Y	U	Y	Y	Y	N	Y	Y
Hwang et al. (2011)[90]	N	Y	U	Y	Y	Y	U	Y	Y
%	0.00	100.00	37.50	100.00	100.00	100.00	0.00	100.00	100.00

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