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RESEARCH

Vaccination against pandemic A/H1N1 2009 influenza in pregnancy and risk of fetal death: cohort study in Denmark

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Abstract

Objective To investigate whether an adjuvanted pandemic A/H1N1 2009 influenza vaccine in pregnancy was associated with an increased risk of fetal death.

Design Nationwide register based cohort study.

Setting Denmark.

Participants All clinically recognised singleton pregnancies that ended between November 2009 and September 2010. Individual level data on exposure to an inactivated AS03 pandemic A/H1N1 2009 influenza vaccine (Pandemrix) and potential confounders were linked to the study cohort using a unique person identifier.

Main outcome measures The primary outcome measure was risk of fetal death (spontaneous abortion and stillbirth combined) in H1N1 vaccinated compared with unvaccinated pregnancies, adjusting for propensity scores. Secondary outcome measures were spontaneous abortion (between seven and 22 weeks' gestation) and stillbirth (after 22 completed weeks' gestation).

Results The cohort comprised 54 585 pregnancies; 7062 (12.9%) women were vaccinated against pandemic A/H1N1 2009 influenza during pregnancy. Overall, 1818 fetal deaths occurred (1678 spontaneous abortions and 140 stillbirths). Exposure to the H1N1 vaccine was not associated with an increased risk of fetal death (adjusted hazard ratio 0.79, 95% confidence interval 0.53 to 1.16), or the secondary outcomes of spontaneous abortion (1.11, 0.71 to 1.73) and stillbirth (0.44, 0.20 to 0.94). Estimates for fetal death were similar in pregnant women with (0.82, 0.44 to 1.53) and without comorbidities (0.77, 0.47 to 1.25).

Conclusion This large cohort study found no evidence of an increased risk of fetal death associated with exposure to an adjuvanted pandemic A/H1N1 2009 influenza vaccine during pregnancy.

Introduction

Pregnant women are at higher risk of morbidity from seasonal influenza infection, and studies from previous pandemics have found that influenza infection in pregnancy is associated with increased mortality.[1-4] Early in the outbreak of pandemic A/H1N1 2009 influenza reports suggested a high risk of morbidity and mortality associated with infection in pregnancy.[5] Consequently, pregnant women were included among the prioritised target groups in the then upcoming H1N1 vaccination campaigns, and at least 322 000 pregnant women were vaccinated in Europe alone.[6 7]

Available evidence on the risks to the fetus of influenza vaccination in pregnancy is, however, limited to only a handful of studies of mainly inactivated seasonal influenza vaccines.[3 8] The few studies dealing with fetal risks associated with pandemic A/H1N1 2009 influenza vaccine in pregnancy have relied on passive pharmacovigilance surveillance or designs without control groups.[7 9-14] Although the data have not indicated an increased risk, the designs of these studies have precluded an adequate analytical approach and they have therefore not

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Maternal comorbidities and drugs included in propensity score, with relevant codes
Characteristics of participants in analysis of spontaneous abortion
Characteristics of participants in analysis of stillbirth
Sensitivity analysis for unmeasured confounder: scenario 1
Sensitivity analysis for unmeasured confounder: scenario 2

been able to exclude risks to the fetus. Thus, comparative analytical studies are needed to accurately assess the safety of H1N1 vaccines.

We carried out a nationwide register based cohort study to investigate whether there was an increased risk of fetal death (spontaneous abortion and stillbirth) after vaccination with an AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine among pregnant women in Denmark.

Methods

We constructed a cohort comprising all singleton pregnancies (live births, stillbirths, and pregnancies with an abortive outcome) in Denmark that ended between 2 November 2009 and 30 September 2010. Among women with several pregnancies in this period, we included only the first. We linked information on H1N1 vaccination and potential confounders to the pregnant women in the cohort using their unique personal identification numbers.[15] The primary outcome was fetal death (spontaneous abortion and stillbirth combined). The secondary outcomes were spontaneous abortion (defined as occurring between the start of week 7 and the end of week 22 of gestation) and stillbirth (defined as delivery of a dead fetus after 22 completed weeks of gestation[16]) analysed separately.

We obtained information on live births and stillbirths from the medical birth register,[17] which contains detailed records on all births in Denmark, including the personal identification number of the parents and the newborn, date of birth, and gestational age. The national patient register contains individual level data on all inpatients and outpatients in Danish hospitals, including personal identification number, dates of contact or admission and discharge, and diagnoses, classified according to the international classification of diseases (10th revision).[18] From this register we obtained information on pregnancies with an abortive outcome: spontaneous abortion (ICD-10 codes O021, O03), ectopic pregnancy (O00), hydatidiform mole (O01), other abnormal products of conception (O020, O028-029), and induced abortion (O04-08). The positive predictive value of a registered diagnosis of spontaneous abortion was 99% when confirmed by review of the medical records.[19] For pregnancies with an abortive outcome, gestational age is indicated by a supplementary code.

The onset of pregnancy was defined as the first day of the last menstrual period and was estimated by subtraction of gestational age from the date of birth or the date of abortive outcome. Recording of gestational age is based on the women's report of the first day of their last menstrual period; the date of the first day of the last menstrual period is usually corrected according to ultrasonography measurements.[20]

We excluded women with a missing or implausible gestational age, an abortive outcome at a gestational age of less than six completed weeks, a diagnosis of an abortive outcome within six weeks before the index pregnancy for pregnancies with an abortive outcome (to avoid double register entries), pregnancy onset before 1 February 2009 or after 6 December 2009 (unlikely to be vaccinated), and those who had received H1N1 vaccination preceding the onset of pregnancy.

Pandemic A/H1N1 2009 influenza vaccine

The Danish H1N1 vaccination programme targeted people with chronic diseases, key government staff, healthcare staff, and pregnant women.[21-23] Vaccination was recommended at any time in pregnancy, including the first trimester after individual assessment, to pregnant women with chronic diseases; vaccination was recommended in the second and third trimesters to pregnant women without comorbidities.[21] The vaccination campaign started on 2 November 2009, and the only vaccine used in Denmark was the monovalent inactivated AS03 adjuvanted split virion H1N1 vaccine (Pandemrix, GlaxoSmithKline Biologicals, Rixensart, Belgium). Pandemic vaccination was provided mainly by general practitioners and also by hospitals and private clinics. We obtained information on vaccination status from a national H1N1 vaccination database, set up at Statens Serum Institut with the aim of nationwide surveillance of vaccination coverage, effectiveness, and safety. During the pandemic, all vaccine providers were mandated, by law, to report and register the personal identification number of the vaccinee and the date of vaccination. Additionally, reporting was obligatory to reimburse costs from the national health insurance. Therefore the database is considered close to complete.

Potential confounders

We included the following variables in a propensity score model: maternal age, county of residence, degree of urbanisation at place of residence, and country of birth (central person register[15]); parity (medical birth register), history of fetal death in siblings (medical birth register and national patient register), selected comorbidities (see supplementary table 1) and number of hospital admissions and outpatient hospital contacts within three years preceding pregnancy (national patient register), and selected drugs (see supplementary table 1) and number of drugs used within six months before pregnancy (national prescription register[24]). The prescription register contains individual level information on all prescriptions filled at all Danish pharmacies, including personal identification number, date of filling the prescription, and the Anatomic Therapeutic Chemical code. Using logistic regression (SAS procedure LOGISTIC; SAS statistical software version 9.2), we estimated a propensity score for each woman as the predicted probability of vaccination conditional on all given covariates. Additionally, we included in the propensity score all two way interactions between covariates, except for comorbidities and drugs. We used mode imputation for variables with missing values. Three different propensity scores were estimated, one for each analysis: fetal death (exposure between pregnancy onset and birth), spontaneous abortion (exposure between pregnancy onset and week 22), and stillbirth (exposure between pregnancy onset and birth). After calculating propensity scores, we excluded women with a non-overlapping probability of vaccine exposure to limit unmeasured confounding from those at the extreme ends of the propensity score distribution.[25]

Statistical analysis

Pregnancies started contributing person time to the cohort on 2 November 2009 or the start of week 7 of gestation, whichever occurred latest, and were followed to fetal death or live birth. We censored pregnancies ending with an abortive outcome other than spontaneous abortion. In the subanalysis of spontaneous abortion, we excluded pregnancies without follow-up in weeks 7-22 of gestation. In the subanalysis of stillbirth, we excluded pregnancies without follow-up after 22 completed weeks of gestation.

We used the 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 was used to estimate hazard ratios with 95% confidence intervals, comparing the hazard rates of fetal death among H1N1 vaccinated and unvaccinated women (SAS procedure

PHREG).[26 27] To assess the validity of the proportional hazards assumption, we performed a Wald test for the interaction between each independent variable and gestational age. If the assessment indicated non-constant effect over time, we included an interaction term in the model. Vaccination was treated as a time dependent variable, thus reflecting vaccination status at a given point in time during pregnancy. Possible confounders were included in the models through propensity scores, classified into distribution fifths.

Common contraindications to vaccination (for example, acute (febrile) illness) may also be associated with an increased risk of fetal death; this would bias results towards a protective effect of vaccination, at least in the period immediately after vaccination. In all analyses we therefore estimated hazard ratios allowing for a two week period immediately after vaccination; this two week period was removed from the main analysis and analysed separately.

In sensitivity analyses, we estimated hazard ratios in women with and without comorbidities by trimester of vaccination without removing the two week period after vaccination from the main analysis, and allowing for a six week period after vaccination (removed from the main analysis). We also estimated the risk of stillbirth, including smoking status and prepregnancy body mass index, in the propensity score (these data, from the medical birth register, were available only for stillborn and live born infants). Additionally, we adjusted models for influenza infection as a time varying variable. Influenza infection was defined as a hospital outpatient contact or inpatient admission for influenza (ICD-10 code J09-11), or a filled prescription for an anti-influenza antiviral (Anatomic Therapeutic Chemical code J05AH01-02). Furthermore, we used the array approach sensitivity analysis described by Schneeweiss to estimate the effect of an unmeasured confounder on the observed estimates.[28]

Results

Fetal death

A total of 75 483 pregnancies ended during 2 November 2009 and 30 September 2010, of which 20 848 were excluded (fig 1⇓). The C statistic for the propensity score for exposure to vaccine between pregnancy onset and birth was 0.62. After exclusion of 50 pregnancies with non-overlapping propensity scores, the final study cohort for the outcome of fetal death comprised 54 585 pregnancies; these ended in 50 552 live births (92.6%), 1678 spontaneous abortions (3.1%), 140 stillbirths (0.3%), and 2215 other abortive pregnancy outcomes (4.1%). A total of 7062 (12.9%) women were vaccinated against H1N1 influenza during pregnancy, with most cohort participants being vaccinated in the second half of November (fig 2⇓). Vaccinated women had higher parity, were slightly older, were slightly more likely to have been born in Denmark, and were more likely to live in the capital, to have had a previous pregnancy ending in fetal death, to have selected comorbidities such as pulmonary disease and diabetes, to have been admitted to hospital in the past three years, to have had an outpatient hospital contact in the past three years, and to have used drugs in the past six months (table 1⇓). A large proportion of women had no registered comorbidities (table 1).

The unadjusted survival curves between vaccinated and unvaccinated women were similar (fig 3⇓). The risk of fetal death associated with H1N1 vaccination in pregnancy was not increased in either unadjusted (hazard ratio 0.82, 95% confidence interval 0.55 to 1.20) or adjusted analyses (0.79, 0.53 to 1.16; table 2⇓). There was a non-significant decreased risk of fetal death in the two week period immediately after vaccination (six exposed cases; adjusted hazard ratio 0.48, 95% confidence interval 0.22 to 1.10).

Spontaneous abortion

From the principal cohort an additional 19 088 pregnancies without follow-up between week 7 and 22 of gestation and 139 pregnancies with non-overlapping propensity scores were excluded (fig 1). The C statistic for the propensity score for exposure to vaccine between pregnancy onset and week 22 was 0.65. Among the 35 408 (2736 (7.7%) vaccinated) contributing pregnancies in this cohort, followed between week 7 and 22 of gestation, 1674 (4.7%) spontaneous abortions occurred. (See supplementary table 2 for the characteristics of participants in this cohort.) No increased risk of spontaneous abortion was observed after H1N1 vaccination in either unadjusted (hazard ratio 1.16, 95% confidence interval 0.74 to 1.80) or adjusted analyses (1.11, 0.71 to 1.73). There was a non-significant decreased risk of spontaneous abortion in the two week period immediately after vaccination (five exposed cases; adjusted hazard ratio 0.47, 95% confidence interval 0.19 to 1.13).

Stillbirth

From the principal cohort, 3905 pregnancies with no follow-up after 22 completed weeks of gestation and 53 pregnancies with non-overlapping propensity scores were excluded (fig 1). The C statistic for propensity score for exposure to vaccine between week 23 and birth was 0.63. Among the 50 677 (7014 (13.8%) vaccinated) contributing pregnancies in this cohort, 139 (0.3%) stillbirths were observed. (See supplementary table 3 for the characteristics of participants in this cohort.) The risk of stillbirth after H1N1 vaccination decreased in both unadjusted (hazard ratio 0.43, 95% confidence interval 0.20 to 0.92) and adjusted analyses (0.44, 0.20 to 0.94; table 2). There was no significantly decreased risk of stillbirth in the two week period after vaccination (one exposed case; adjusted hazard ratio 0.58, 95% confidence interval 0.08 to 4.18).

Sensitivity analyses

The risk of fetal death was estimated in pregnant women with and without any registered predefined comorbidities: hazard ratios were similar in these two groups (table 3⇓). In analyses of fetal death according to trimester of vaccination, the risk associated with vaccination in the first trimester was not significantly increased (table 3); there was a significantly decreased risk associated with vaccination in the second trimester and no significantly increased risk associated with vaccination in the third (table 3). In analyses of all three outcomes without removing the two week period after vaccination and also when increasing the duration of the period to six weeks, the hazard ratios tended towards zero (table 3). For the analysis of stillbirth, the estimate was not changed by including smoking and body mass index in the propensity score (table 3). When influenza infection was included as a time varying covariate in the models, the hazard ratio did not change for any of the three outcomes (table 3). Finally, the potential effect of an unmeasured confounder was modelled on the fetal death outcome. A confounder that would mask a true risk associated with vaccination would be of primary concern; it was therefore assumed that those receiving vaccination had a lower prevalence of an unmeasured confounder that was associated with increased risk of fetal death (see supplementary table 4). For example, if 20% of the non-vaccinated and 10% of the vaccinated women had the confounder, and the

confounder increased the relative risk of fetal death by 3, the observed estimate of 0.8 would be biased by 14% and a confounder adjusted estimate would be 0.9. Assuming a greater difference in prevalence of confounders between groups, 20% in the non-vaccinated and 0% in the vaccinated, and a confounder associated relative risk of 3, the observed estimate of 0.8 would be biased by 29% and a confounder adjusted estimate would be 1.1. Assuming a larger confounder associated relative risk of 5.5 with prevalence of confounders of 20% and 10%, the observed estimate of 0.8 would be biased by 24% and a confounder adjusted estimate would be 1.0. A similar but alternative scenario was also modelled, assuming a higher prevalence of a protective unmeasured confounder among the vaccinated women (see supplementary table 5). For example, if 50% of the vaccinated women and 20% of the non-vaccinated women had a confounder with a relative risk for fetal death of 0.4, the observed estimate of 0.8 would be biased by 20% and a confounder adjusted estimate would be 1.0.

Discussion

In this large nationwide cohort study, vaccination with an AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine in pregnancy was not associated with an increased risk of the primary composite outcome of fetal death, or its components spontaneous abortion and stillbirth. Given the upper limit of the confidence interval, these data allowed the exclusion of a 17% increased risk of fetal death, a 74% increased risk of spontaneous abortion, and any risk of stillbirth associated with vaccination. Results were similar in healthy pregnant women and those with comorbidities.

Comparison with other studies

Previous reports on the fetal safety of H1N1 vaccination, although reassuring, have been non-analytical and limited in size. The Vaccine Adverse Events Reporting System in the United States received 131 reports of pregnancy specific events in women receiving non-adjuvanted H1N1 vaccines, with spontaneous abortion (n=95) and stillbirth (n=18) representing the most common events[11]; estimated reporting rates were considerably lower than expected rates. A review of spontaneous reports received by the European Medicines Agency identified 130 pregnancy related outcomes, including 57 abortions and 49 intrauterine deaths or stillbirths. Given the number of vaccinated women and background rates, this, in addition to data provided by the manufacturers, was not considered to indicate increased risk.[7 14] Similarly, pharmacovigilance reports from Taiwan and France found a lower than expected number of reported events.[10 13] These studies, based on data from passive surveillance systems, have limitations; importantly, although they can generate risk signals, they cannot exclude risks. Two descriptive cohort studies have been published. In a post-authorisation study of the AS03 adjuvanted H1N1 vaccine in the United Kingdom, pregnancy outcomes from 265 vaccinated pregnant women were reported[12]; the prevalence of spontaneous abortions and stillbirths was comparable to expected rates. A French study had outcome data for 569 pregnant women receiving a non-adjuvanted H1N1 vaccine; rates of fetal death (unclear definition) and stillbirth were not higher than in other surveys.[9] Thus our cohort study confirms the findings from limited previous reports and expands on these findings by providing the first detailed comparative analysis of the risk of fetal death associated with H1N1 vaccination.

Pandemrix is a split virion vaccine produced from the A/California/7/2009 (H1N1)v-like strain and contains an AS03 adjuvant (composed of squalene, DL-α-tocopherol, and polysorbate 80). Our results are principally applicable to this particular vaccine. Additionally, we believe that results are generalisable to non-adjuvanted vaccines produced from this virus strain (although these generally have a higher content of the antigen haemagglutinin) but not to vaccines with other adjuvants. Because of the antigenic differences, it is less clear whether our results add to the available data on safety of fetuses when using non-adjuvanted seasonal influenza vaccines, for which limited information on risk of fetal death is available.[3 8]

Strengths and limitations of the study

Strengths of this study include its size and its comprehensive design, with register linkage of individual level data. Information on H1N1 vaccination was obtained through a nationwide database, to which reporting was mandatory. This eliminates recall bias, ensures completeness, and improves the accuracy of information on timing of vaccination compared with self reported exposure. Registration of births is mandatory in the medical birth register; it is therefore unlikely that we missed any significant number of births. Most cases of spontaneous abortion in Denmark are likely to be managed by hospital doctors. According to national guidelines for pregnancy care, the investigation of early pregnancy bleeds includes ultrasonography as a central part, and guidelines from the Danish Society of Obstetrics and Gynecology indicate that the diagnosis of spontaneous abortion requires confirmation by ultrasonography.[29 30] An ultrasound examination on this indication could only be carried out by an obstetrician-gynaecologist. We therefore believe that our hospital based strategy for the detection of spontaneous abortions was close to complete. We excluded registered spontaneous abortions with less than six completed weeks of gestation—that is, early pregnancy loss; only a limited proportion of early pregnancy losses are recognised clinically, therefore inclusion of this time period in the analyses would have introduced outcome misclassification.

A relatively small proportion (13%) of pregnant women in this nationwide Danish cohort was vaccinated against pandemic A/H1N1 2009 influenza. This rate is similar to that in some countries but contrasts with the rate in others—for example, the estimated vaccine coverage among pregnant women was 8% in Germany, 23% in France, and 40% in the United States.[31-33] The reason for the low vaccine uptake in Denmark possibly relates to the fact that the 2009-10 season was the first time that pregnant women without comorbidities were included among the target groups for influenza vaccination.

Although we adjusted for many potential confounders, there might have been differences between vaccinated and unvaccinated women associated with both exposure and outcome that we could not measure. Of concern would be factors that could have obscured a risk of fetal death associated with vaccination. Such factors would have to be common or strongly associated with both vaccination and reduced risk of fetal death. A few factors might meet these criteria. Although vaccinated women in our study had a somewhat higher rate of comorbidities compared with unvaccinated women, a large proportion of the cohort did not have any registered comorbidities. A healthy vaccinee effect, conferred by, for example, unmeasured factors such as diet, exercise, educational level, or socioeconomic class, that masks a risk of fetal death among vaccinated women cannot be excluded. However, given our observed main estimate of 0.79, the difference in prevalence between the groups would have to be large and the association with reduced risk of fetal death strong to obscure a true increased risk associated with

vaccination. Additionally, we tackled the problem of a healthy vaccinee effect partly by adjusting for smoking and body mass index, important risk factors for stillbirth,[34] in a sensitivity analysis; this did not change the estimates. Because most people who contract influenza never seek healthcare, the data sources used in our study did not allow for a precise adjustment for influenza infection. However, in sensitivity analyses where we adjusted for influenza infection, defined as the registration of a hospital diagnosis for influenza or the filling of a prescription for an anti-influenza antiviral drug, the main results were unchanged. It should, however, be noted that this definition of influenza could not detect those cases of influenza infection where patients did not seek hospital care or cases where patients were not prescribed antivirals. In a scenario where a true increased risk of fetal death associated with vaccination exists, an increased risk of fetal death conferred by H1N1 infection itself among unvaccinated women might have biased results towards the null. However, given the 5% cumulative incidence of H1N1 infection in Denmark,[35] we reason that confounding by infection strong enough to contradict the conclusion of this study is unlikely. Finally, the absence of a strong influence from unmeasured confounding is supported by our sensitivity analysis, which showed that when adjusting for a hypothetical confounder with high prevalence among unvaccinated women and strong association with the outcome, the confounder adjusted estimate for the association between vaccination and fetal death would not be far over 1. This sensitivity analysis indicated similar results when assuming a scenario where vaccinated women had a higher prevalence of an unmeasured confounder that was strongly protective.

Several factors need to be considered when interpreting our finding of a significantly decreased risk of stillbirth associated with vaccination against H1N1 influenza. Although H1N1 infection is associated with an increased risk of stillbirth,[36] and a protective effect of vaccination therefore may seem plausible, the objective of our study was to investigate a possible increased risk of adverse events. Additionally, the analysis of stillbirth was a prespecified secondary outcome and based on a small number of cases. A chance finding therefore represents a possibility. Thus the result from this analysis should be viewed as hypothesis generating and needs investigation in other studies before any conclusions on protective effects are drawn.

Conclusions

In conclusion, this nationwide study is the first to analytically investigate fetal risks associated with vaccination against pandemic A/H1N1 2009 influenza in pregnancy. In a cohort of more than 50 000 pregnancies there was robust evidence for the safety of the AS03 adjuvanted H1N1 vaccine with respect to the composite outcome of fetal death and its components, spontaneous abortion and stillbirth. Additional studies of other fetal outcomes are needed to establish the complete safety profile of this vaccine in pregnancy.

Contributors: All authors contributed to the conception and design of the study, the analysis and interpretation of the study results, and critical revision of the manuscript. BP and AH drafted the manuscript. HS conducted the statistical analyses. HS, TGK, HDE, and AH acquired the data. AH and MM supervised the study. AH is the guarantor. All authors approved the final version of the manuscript for submission.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: BP had support from the Danish Medical Research Council and Lund University for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: This study was approved by the Danish Data Protection Agency. Ethical approval is not required for register based research in Denmark.

Data sharing: No additional data available.

1 Dodds L, McNeil SA, Fell DB, Allen VM, Coombs A, Scott J, et al. Impact of influenza exposure on rates of hospital admissions and physician visits because of respiratory illness among pregnant women. *CMAJ* 2007;176:463-8.
2 Harris JW. Influenza occurring in pregnant women. *JAMA* 1919;14:978-80.
3 Mak TK, Mangtani P, Leese J, Watson JM, Pfeifer D. Influenza vaccination in pregnancy: current evidence and selected national policies. *Lancet Infect Dis* 2008;8:44-52.
4 Greenberg M, Jacobziner H, Pakter J, Weisel BAG. Maternal mortality in the epidemic of asian influenza, New York City, 1957. *Am J Obstet Gynecol* 1957;897-902.
5 Jamieson DJ, Honein MA, Rasmussen SA, Williams JL, Swerdlow DL, Biggerstaff MS, et al. H1N1 2009 influenza virus infection during pregnancy in the USA. *Lancet* 2009;374:451-8.
6 Luteijn JM, Dolk H, Marnoch GJ. Differences in pandemic influenza vaccination policies for pregnant women in Europe. *BMC Public Health* 2011;11:819.
7 European Medicines Agency. Eleventh pandemic pharmacovigilance weekly update. 2010. www.ema.europa.eu/docs/en_GB/document_library/Report/2010/02/WC500073992.pdf.
8 Tamma PD, Ault KA, del Rio C, Steinhoff MC, Halsey NA, Omer SB. Safety of influenza vaccination during pregnancy. *Am J Obstet Gynecol* 2009;201:547-52.
9 Omon E, Damase-Michel C, Hurault-Delarue C, Lacroix I, Montastruc JL, Oustric S, et al. Non-adjuvanted 2009 influenza A (H1N1)v vaccine in pregnant women: the results of a French prospective descriptive study. *Vaccine* 2011;29:9649-54.
10 Huang WT, Chen WC, Teng HJ, Huang WI, Huang YW, Hsu CW, et al. Adverse events following pandemic A (H1N1) 2009 monovalent vaccines in pregnant women—Taiwan, November 2009-August 2010. *PLoS One* 2011;6:e23049.
11 Moro PL, Broder K, Zheteyeva Y, Revzina N, Tepper N, Kissin D, 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;205:473-9.
12 Tavares F, Nazareth I, Monegal JS, Kolte I, Verstraeten T, Bauchau V. Pregnancy and safety outcomes in women vaccinated with an AS03-adjuvanted split virion H1N1 (2009) pandemic influenza vaccine during pregnancy: a prospective cohort study. *Vaccine* 2011;29:6358-65.
13 Lacroix I, Damase-Michel C, Kreft-Jais C, Castot A, Montastruc JL. 2009 H1N1 influenza vaccines in pregnant women: the French pharmacovigilance survey. *Vaccine* 2011;29:1357-8.
14 European Medicines Agency. Twenty-second pandemic pharmacovigilance update. 2010. www.ema.europa.eu/docs/en_GB/document_library/Report/2010/08/WC500095870.pdf.
15 Pedersen CB, Gotzsche H, Moller JO, Mortensen PB. The Danish Civil Registration System. A cohort of eight million persons. *Dan Med Bull* 2006;53:441-9.
16 Danish National Board of Health. Vejledning om kriterier for levende-og dødsfødsel mv. [In Danish.] 31 Aug 2005. www.sst.dk/publ/Publ2005/KOT/Vejl_levende_doed/Vejl_kriterier_levende.pdf.
17 Knudsen LB, Olsen J. The Danish Medical Birth Registry. *Dan Med Bull* 1998;45:320-3.
18 Andersen TF, Madsen M, Jorgensen J, Mellemkjoer L, Olsen JH. The Danish National Hospital Register. A valuable source of data for modern health sciences. *Dan Med Bull* 1999;46:263-8.
19 Lohse SR, Farkas DK, Lohse N, Skouby SO, Nielsen FE, Lash TL, et al. Validation of spontaneous abortion diagnoses in the Danish National Registry of Patients. *Clin Epidemiol* 2010;2:247-50.
20 Jorgensen FS. [Ultrasonography of pregnant women in Denmark 1999-2000. Description of the development since 1980-1990]. *Ugeskr Laeger* 2003;165:4409-15.
21 Danish National Board of Health. Anbefalinger for vaccination af personer i risiko for alvorlig sygdom på grund af A(H1N1)v infektion. [In Danish.] 23 Oct 2009. www.sst.dk/~/media/Sundhed%20og%20forebyggelse/Smitsomme%20sygdomme/Influenza/Vaccination_lister/Anbefalinger_vaccination_risikogrupper23okt.ashx.
22 Danish National Board of Health. Vaccination mod influenza A (H1N1). Tabel over nøglepersoner i kritiske funktioner, total. [In Danish.] 29 Sep 2009. www.sst.dk/~/media/Sundhed%20og%20forebyggelse/Smitsomme%20sygdomme/Influenza/Vaccination_lister/Tabel_vaccination_noeglepersoner_total.ashx.
23 Danish National Board of Health. Vaccination mod influenza A (H1N1). Tabel over sundheds-og plejepersonale. [In Danish] 29 Sep 2009. www.sst.dk/~/media/Sundhed%20og%20forebyggelse/Smitsomme%20sygdomme/Influenza/Vaccination_lister/sundhedspersonale_vaccination_liste20090929.ashx.
24 Kildemoes HW, Sorensen HT, Hallas J. The Danish National Prescription Registry. *Scand J Public Health* 2011;39:38-41.
25 Sturmer T, Rothman KJ, Avorn J, Glynn RJ. Treatment effects in the presence of unmeasured confounding: dealing with observations in the tails of the propensity score distribution—a simulation study. *Am J Epidemiol* 2010;172:843-54.
26 Clayton D, Hills M. Cox's regression analysis. In: Clayton D, Hills M, eds. Statistical models in epidemiology. Oxford University Press, 2001:298-306.
27 Andersen AM, Vastrup P, Wohlfahrt J, Andersen PK, Olsen J, Melbye M. Fever in pregnancy and risk of fetal death: a cohort study. *Lancet* 2002;360:1552-6.

What is already known on this topic

Pregnant women infected with pandemic A/H1N1 2009 influenza were at increased risk of morbidity, mortality, and poor pregnancy outcomes

In many countries, the H1N1 vaccination campaigns included pregnant women among the target groups

What this study adds

In a cohort of over 50 000 pregnancies in Denmark, there was no increased risk of the composite outcome of fetal death and its components, spontaneous abortion and stillbirth, associated with exposure to an adjuvanted pandemic A/H1N1 2009 influenza vaccine

28 Schneeweiss S. Sensitivity analysis and external adjustment for unmeasured confounders in epidemiologic database studies of therapeutics. *Pharmacoepidemiol Drug Saf* 2006;15:291-303.
29 Danish National Board of Health. Anbefalninger for svangreomsorgen. [In Danish.] 2009. www.sst.dk/publ/Publ2009/CFF/gravide/Svangreomsorgen.pdf.
30 Danish Society of Obstetrics and Gynecology. Spontan abort og missed abortion i første trimester. [In Danish.] May 2011. www.dsog.dk/hindsgavl/Spontan%20abort%20og%20missed%20abortion%20i%20frste%20trimester.pdf.
31 Influenza vaccination coverage among pregnant women—29 states and new york city, 2009-10 season. *MMWR Morb Mortal Wkly Rep* 2012;61:113-8.
32 Bone A, Guthmann JP, Nicolau J, Levy-Bruhl D. Population and risk group uptake of H1N1 influenza vaccine in mainland France 2009-2010: results of a national vaccination campaign. *Vaccine* 2010;28:8157-61.
33 Walter D, Bohmer MM, Heiden M, Reiter S, Krause G, Wichmann O. Monitoring pandemic influenza A(H1N1) vaccination coverage in Germany 2009/10—results from thirteen consecutive cross-sectional surveys. *Vaccine* 2011;29:4008-12.
34 Flenady V, Koopmans L, Middleton P, Froen JF, Smith GC, Gibbons K, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. *Lancet* 2011;377:1331-40.
35 Molbak K, Widgren K, Jensen KS, Ethelberg S, Andersen PH, Christiansen AH, et al. Burden of illness of the 2009 pandemic of influenza A (H1N1) in Denmark. *Vaccine* 2011;29(Suppl 2):B63-9.
36 Pierce M, Kurinczuk JJ, Spark P, Brocklehurst P, Knight M. Perinatal outcomes after maternal 2009/H1N1 infection: national cohort study. *BMJ* 2011;342:d3214.

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Tables

Table 1| Characteristics of women vaccinated and not vaccinated against pandemic A/H1N1 2009 influenza during pregnancy, nationwide cohort, Denmark. Values are numbers (percentages) unless stated otherwise

Characteristics	Unvaccinated (n=47 523)	Vaccinated (n=7062)	P value*
Pregnancy outcome:			
Live birth	43 546 (92)	7006 (99)	
Stillbirth	132 (0.3)	8 (0.1)	
Spontaneous abortion	1653 (3)	25 (0.4)	
Ectopic pregnancy	88 (0.2)	3 (<0.1)	
Hydatidiform mole	7 (<0.1)	0 (0)	
Other abnormal products of conception	107 (0.2)	1 (<0.1)	
Induced abortion	1990 (4)	19 (0.3)	
Mean (SD) age at onset of pregnancy	30.0 (5.2)	30.9 (4.7)	<0.01
Age group at onset of pregnancy†:			
<20	1454 (3)	90 (1)	<0.01
20-24	6663 (14)	691 (10)	
25-29	15 582 (33)	2199 (31)	
30-34	15 916 (33)	2716 (38)	
35-39	6759 (14)	1193 (17)	
≥40	1149 (2)	173 (2)	
County of residence‡:			
Capital region	16 946 (36)	2770 (39)	<0.01
Sjælland region	5956 (13)	672 (10)	
Syddanmark region	9465 (20)	1433 (20)	
Midtjylland region	10 722 (23)	1573 (22)	
Nordjylland region	4434 (9)	614 (9)	
Degree of urbanisation§:			
≤49 inhabitants/km^2	2373 (5)	293 (4)	
50-99 inhabitants/km^2	12 940 (27)	1690 (24)	<0.01
100-199 inhabitants/km^2	9907 (21)	1328 (19)	
≥200 inhabitants/km^2	5147 (11)	920 (13)	
Copenhagen suburbs	8899 (19)	1415 (20)	
Copenhagen	8257 (17)	1416 (20)	
Place of birth¶:			
Denmark	39 609 (83)	6037 (85)	<0.01
Europe	2152 (5)	330 (5)	
Other	5762 (12)	695 (10)	
Month of pregnancy onset, 2009:			
February	3447 (7)	399 (6)	<0.01
March	3771 (8)	1025 (15)	
April	3832 (8)	1094 (15)	
May	4031 (8)	1290 (18)	
June	3854 (8)	1132 (16)	
July	3908 (8)	990 (14)	
August	4713 (10)	714 (10)	
September	5896 (12)	231 (3)	
October	6313 (13)	125 (2)	
November	6514 (14)	61 (1)	
December	1244 (3)	1 (<0.1)	

Table 1 (continued)

Characteristics	Unvaccinated (n=47 523)	Vaccinated (n=7062)	P value*
Parity at onset of pregnancy:			
0	22 172 (47)	2851 (40)	<0.01
1	16 498 (35)	2890 (41)	
2	6617 (14)	1011 (14)	
≥3	2236 (5)	310 (4)	
History of fetal death	4752 (10)	823 (12)	<0.01
Morbidities and drugs**:			
Pulmonary disease/antiobstructive inhalants	1736 (4)	474 (7)	<0.01
Cardiovascular disease/cardiovascular drugs	1235 (3)	279 (4)	<0.01
Haematological disease	418 (1)	92 (1)	<0.01
Diabetes/antidiabetic drugs	703 (1)	195 (3)	<0.01
Neurological disease	1031 (2)	212 (3)	<0.01
Liver and kidney disease	286 (1)	55 (1)	0.08
Rheumatic disease	222 (0.5)	57 (1)	<0.01
Inflammatory bowel disease/intestinal anti-inflammatory agents	418 (1)	93 (1)	<0.01
Obesity	2274 (5)	400 (6)	<0.01
Immunodeficiency/immunosuppressants	65 (0.1)	30 (0.4)	<0.01
Disorders of female pelvic organs/genital tract	5187 (11)	920 (13)	<0.01
Hospital contact for injury or poisoning	10 612 (22)	1501 (21)	0.04
Antidepressants	2323 (5)	407 (6)	<0.01
Antiepileptics	292 (1)	48 (1)	0.52
Drugs for peptic ulcer/gastroesophageal reflux	1250 (3)	239 (3)	<0.01
Contraceptive pills	11 066 (23)	1510 (21)	<0.01
Drugs for in vitro fertilisation	3224 (7)	559 (8)	<0.01
Thyroid hormones	475 (1)	96 (1)	<0.01
Systemic corticosteroids	593 (1)	129 (2)	<0.01
Non-steroidal anti-inflammatory drugs	3984 (8)	652 (9)	0.02
Opioids	970 (2)	174 (2)	0.02
Systemic antibacterial agents	10 710 (23)	1799 (25)	<0.01
Hospital admissions in past 3 years:			
0	23 350 (49)	3112 (44)	<0.01
1-2	20 517 (43)	3301 (47)	
3-4	2813 (6)	479 (7)	
≥5	843 (2)	170 (2)	
Outpatient hospital contacts in past 3 years:			
0	15 648 (33)	2034 (29)	<0.01
1-2	18 005 (38)	2648 (37)	
3-4	8705 (18)	1388 (20)	
≥5	5165 (11)	992 (14)	
Drugs used in past 6 months:			
0	15 769 (33)	1971 (28)	<0.01
1-2	20 527 (43)	3033 (43)	
3-4	7460 (16)	1267 (18)	
≥5	3767 (8)	791 (11)	

Because of rounding, percentages may not total 100.

*χ^2 test for categorical values and *t* test for continuous values.

†Missing values: 2 (<0.1%).

‡Missing values: 1504 (2.8%).

Table 1 (continued)

Characteristics	Unvaccinated (n=47 523)	Vaccinated (n=7062)	P value*

§Missing values: 1505 (2.8%).

¶Missing values: 132 (0.2%).

**Comorbidities registered in past three years, and drugs registered in past six months.

Table 2| Association between vaccination against pandemic A/H1N1 2009 influenza in pregnancy and risk of fetal death in nationwide cohort of 54 585 pregnancies in Denmark

				Hazard ratio (95% CI)	
Outcome	**No of women**	**Fetal years at risk**	**No of events**	**Unadjusted**	**Adjusted***
Fetal death:					
Unvaccinated	47 523	19 578	1785	1 (reference)	1 (reference)
Vaccinated	7062	1758	27	0.82 (0.55 to1.20)	0.79 (0.53 to 1.16)
Spontaneous abortion:					
Unvaccinated	32 672	6835	1649	1 (reference)	1 (reference)
Vaccinated	2736	211	20	1.16 (0.74 to 1.80)	1.11 (0.71 to 1.73)
Stillbirth:					
Unvaccinated	43 663	12 728	131	1 (reference)	1 (reference)
Vaccinated	7014	1547	7	0.43 (0.20 to 0.92)	0.44 (0.20 to 0.94)

*Adjusted for propensity scores.

Table 3| Sensitivity analyses of association between vaccination against pandemic A/H1N1 2009 influenza in pregnancy and risk of fetal death

Adverse pregnancy outcomes	Adjusted hazard ratio (95% CI)*
Risk of fetal death according to comorbidity status†:	
Comorbidity	0.82 (0.44 to 1.53)
No comorbidity	0.77 (0.47 to 1.25)
Risk of fetal death according to trimester of vaccination:	
First	0.96 (0.63 to 1.47)
Second	0.49 (0.26 to 0.93)
Third	0.23 (0.03 to 1.61)
Main analysis without two week period after vaccination:	
Fetal death	0.70 (0.50 to 1.00)
Spontaneous abortion	0.87 (0.58 to 1.30)
Stillbirth	0.45 (0.22 to 0.93)
Main analysis allowing for six week period after vaccination:	
Fetal death	0.62 (0.35 to 1.10)
Spontaneous abortion	1.05 (0.50 to 2.21)
Stillbirth	0.40 (0.16 to 0.98)
Body mass index and smoking status included in propensity score:	
Stillbirth	0.45 (0.21 to 0.96)
Main analysis adjusted for influenza infection‡:	
Fetal death	0.78 (0.53 to 1.16)
Spontaneous abortion	1.11 (0.71 to 1.73)
Stillbirth	0.44 (0.20 to 0.94)

*Adjusted for propensity scores.

†Comorbidity if registered with any of the diagnoses listed in table 1, with exception of disorders of female pelvic organ/genital tract and hospital contact for injury and poisoning.

‡Registered diagnosis of influenza during hospital contact or admission, or filled prescription for anti-influenza antiviral drug.

Figures

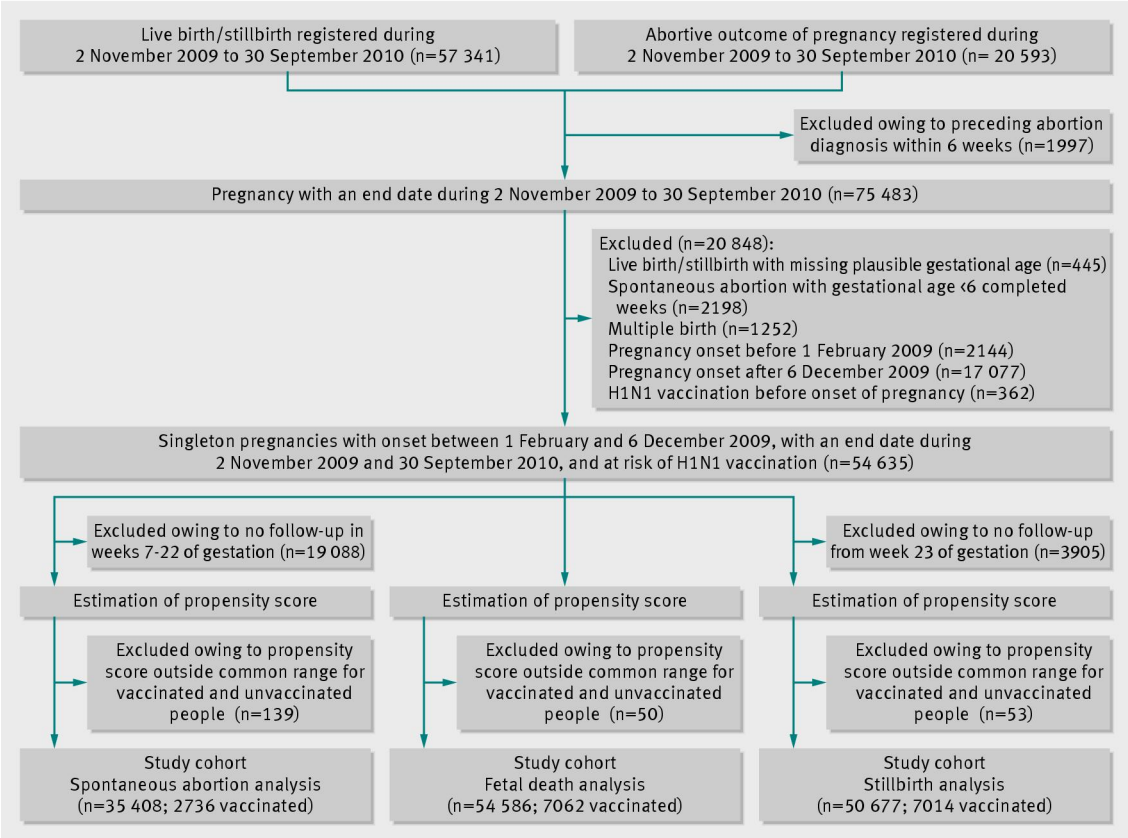

Fig 1 Flow of women through study

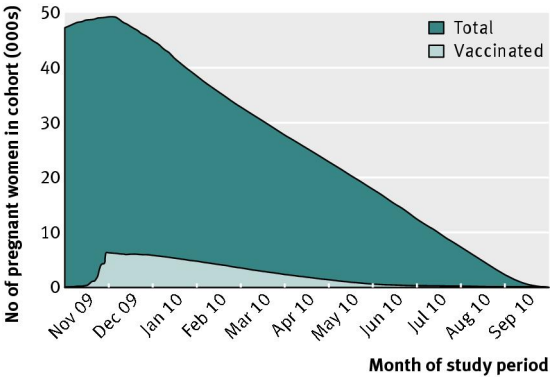

Fig 2 Cumulative number of pregnant women in cohort as well as cumulative number of pregnant women vaccinated against pandemic A/H1N1 2009 influenza throughout study period

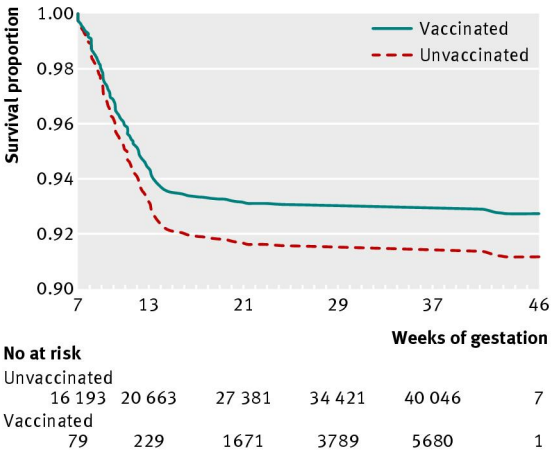

Fig 3 Unadjusted Kaplan-Meier curves for primary outcome of fetal death according to vaccination status. Scale on y axis is restricted to 0.90-1.00