Maternal Immune Response and Neonatal Seroprotection From a Single Dose of a Monovalent Nonadjuvanted 2009 Influenza A(H1N1) Vaccine A Single-Group Trial

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Annals of Internal Medicine | ORIGINAL RESEARCH

Maternal Immune Response and Neonatal Seroprotection From a Single Dose of a Monovalent Nonadjuvanted 2009 Influenza A(H1N1) Vaccine

A Single-Group Trial

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Background: Pregnant women and infants who get influenza are at increased risk for severe illness.

Objective: To evaluate the immunogenicity and transplacental antibody transfer of 2009 pandemic influenza A(H1N1) vaccine administered during pregnancy.

Design: Prospective, multicenter, single-group clinical trial. (Clinical Trials.gov registration number: NCT01024400)

Setting: Five level-3 perinatal centers in France.

Patients: 107 pregnant women between $22^{0/7}$ and $32^{0/7}$ weeks of gestation.

Intervention: An intramuscular dose of a nonadjuvanted H1N1 vaccine that contained 15 mcg of hemagglutinin.

Measurements: Proportion of women with an influenza antibody titer of 1:40 or greater at days 21 and 42 after vaccination, delivery, and 3 months after delivery. Seroconversion rate, fold increase in the geometric mean titer 21 days after vaccination, and proportion of neonates with an antibody titer of 1:40 or greater at birth were also assessed.

Results: At baseline, 19% of the women had an antibody titer of 1:40 or greater. At day 21, 98% of the women had an antibody titer of 1:40 or greater, the seroconversion rate was 93%, and the fold increase in geometric mean titer was 67.4. At day 42, delivery, and 3 months after delivery, 98%, 92%, and 90% of the women, respectively, had an antibody titer of 1:40 or greater. Ninety-five percent of the cord serum samples obtained from 88 neonates showed an antibody titer of 1:40 or greater. The median neonate–mother antibody titer ratio was 1.4.

Limitations: Only healthy pregnant women were selected. Data on hemagglutination inhibition antibody titers of infants were reported only at birth.

Conclusion: A single dose of a nonadjuvanted influenza A(H1N1) vaccine with 15 mcg of hemagglutinin triggered a strong immune response in pregnant women and a high rate of neonatal seroprotection.

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* For a list of Inserm C09-33 PREFLUVAC Study Group members, see the **Appendix** (available at www.annals.org).

Pregnant women are considered to be at high risk for severe illness from influenza infection (1). During influenza season, pregnant women who have an underlying medical condition, are older, or are exposed to the virus in their third trimester are more likely to be hospitalized for respiratory illness than nonpregnant women (2, 3). Pregnant women also have a higher risk for death during influenza pandemics than during nonpandemic years. The mortality rate associated with infection was more than 50% among pregnant women with pneumonia during the 1918 "swine flu" pandemic, and 50% of the women of childbearing age who died of influenza during the 1957 "Asian influenza" pandemic were pregnant (4).

Studies of inactivated seasonal influenza vaccines in pregnant women have shown identical antibody responses in pregnant and nonpregnant women, high cord antibody levels to influenza in neonates born to mothers immunized during pregnancy, and no safety concerns (5). In a prospective, randomized, controlled trial (6), administering influenza vaccine in the third trimester reduced proven influenza illness by 63% in infants up to 6 months of age and avoided approximately one third of all febrile respiratory illnesses with fever in mothers and young infants.

Immunizing pregnant women against influenza benefits both mothers and infants. The World Health Organization (WHO) recommends that all pregnant women be immunized during the influenza season (7). In the United

See also:

Print
Editors' Notes 734
Summary for Patients I-52

Web-Only
Appendix
Appendix Figure
Conversion of graphics into slides

Context

Pregnant women have increased morbidity and mortality from 2009 influenza A(H1N1) infection.

Contribution

Nearly all women who received a single dose of a nonadjuvanted 2009 influenza A(H1N1) vaccine in their second and third trimesters of pregnancy had antibody titers that were considered protective. Antibody titers in cord blood samples from 95% of the infants were also considered protective.

Caution

The study was not powered to assess clinical outcomes.

Implication

A single dose of influenza vaccine administered to women during pregnancy should protect mothers and their newborns from 2009 influenza A(H1N1).

—The Editors

States and Canada, vaccinating healthy pregnant women is recommended regardless of trimester (1). A survey of 29 European countries (8) reported that 8 countries recommended vaccination for pregnant women. In France, seasonal influenza vaccination can be done at any time during pregnancy but is recommended only in pregnant women with an underlying condition (9).

The emergence of influenza A(H1N1) infection in Mexico and Australia in early 2009 increased awareness and concern worldwide. In June 2009, WHO raised the pandemic alert level to 6, the highest level (10). In August 2009, researchers from the Centers for Disease Control and Prevention (CDC) (11) reported that 6 of 45 patients (13%) who died of 2009 influenza A(H1N1) between mid-April and mid-June were pregnant women. A CDC survey (12) confirmed this disproportionate risk for death from 2009 influenza A(H1N1) in pregnant women, which led to their designation as a top-priority group to receive the 2009 H1N1 vaccine. French authorities recommended vaccination with a single dose of a nonadjuvanted H1N1 vaccine for all pregnant women after the first trimester.

We originally planned to evaluate the protection conferred by 2 doses of vaccine administered 21 days apart. However, after the French authorities' recommendations, we opted for a single-group study to evaluate the immunogenicity and transplacental antibody transfer of a nonadjuvanted H1N1v vaccine given in the second or third trimester.

METHODS

This phase 2, prospective, single-group clinical trial was performed in 5 level-3 perinatal centers in France. Women aged 18 to 45 years were eligible if they were pregnant and between $22^{0/7}$ and $32^{0/7}$ weeks of gestation. We excluded women who were allergic to eggs or other components of the vaccine or had a history of severe reaction after influenza vaccine; virologically documented influenza A(H1N1) in the past 6 months; a febrile episode in the week before vaccination; known HIV, hepatitis B virus, or hepatitis C virus infection; an organ transplant; cancer in the past 3 years; multiple sclerosis; history of the Guillain–Barré syndrome; another vaccination in the 3 weeks before study entry or planned in the month after the vaccination; a history of cardiac disease, chronic liver disease, diabetes before pregnancy, or premature delivery or eclampsia; or a fetus with morphologic abnormalities. Women who received systemic corticosteroids, immunotherapy, chemotherapy, anticoagulants, immunoglobulin, or a blood transfusion in the 3 months before enrollment were excluded. To avoid including cases of fetal malformation, women were included after the ultrasonography performed between $21^{0/7}$ and $23^{0/7}$ weeks of gestation.

To balance the study samples for pregnancy duration, women were enrolled in 2 groups of similar size: $22^{0/7}$ to $26^{6/7}$ weeks of gestation and $27^{0/7}$ to $32^{0/7}$ weeks of gestation.

Intervention

Women received 1 injection of a monovalent, inactivated split-virion influenza A(H1N1) vaccine (Sanofi Pasteur, Lyon, France). The vaccine seed virus was prepared from the reassortant virus NYMC X-179A (New York Medical College, New York, New York) generated from the influenza A/California/7/2009 strain as recommended by WHO (13). The seed virus was propagated on embryonated eggs. The vaccine was formulated to contain 15 mcg of hemagglutinin per 0.5-mL dose and was injected intramuscularly into the deltoid.

Outcomes and Follow-up

To assess hemagglutination inhibition (HI) antibody titers and perform microneutralization testing, we planned to obtain maternal blood samples on day 0 (before vaccination), after vaccination on days 21 and 42, at delivery, and at 3 months after delivery. Standard biochemical and blood tests were planned for days 0, 21, and 42 after vaccination. At delivery, cord serum samples were recovered to assess the transplacental transfer of HI antibodies.

At 1 month and 6 months after giving birth, the mothers were contacted by phone to answer questions about their infants, the onset of influenza-like symptoms, and hospitalization since the birth (**Appendix Figure**, available at www.annals.org).

Each woman gave written informed consent before enrollment. Our protocol was conducted in accordance with the Declaration of Helsinki and French law for biomedical research and was approved by the Ile de France III Ethics Committee (Paris, France). An independent adjudication committee, comprising an independent pediatrician, neonatologist, and obstetrician, reviewed the data concerning congenital malformations and hospitalization during the first 4 weeks after birth. Members of the committee had to

determine whether these events were linked or possibly linked to vaccination or whether the link was impossible to assess.

Adverse Event Assessment

Information on local and general reactions was collected during the 30 minutes after vaccination. Pregnant women were then provided with diary cards to record the occurrence and severity of specified local reactions at the injection site (pain, erythema, induration, edema, or ecchymosis), specified general reactions (asthenia, fever, sweating, chills, arthralgia, myalgia, or headache), and any adverse events during the study. Data on adverse events of special interest, including neurologic disorders (such as the Guillain–Barré syndrome, Bell palsy, seizures or convulsions, or encephalitis), immune system disorders (such as autoimmune diseases or anaphylaxis), or cases of influenza A(H1N1) (vaccine failures) were collected. Patients who had an influenza-like illness, defined as an oral temperature greater than 37.8 °C with at least 1 influenza-like symptom, were asked to provide nasal and throat swab specimens for virologic testing. Information on specific adverse events related to pregnancy (preterm delivery, threatened preterm delivery, preterm rupture of the membranes, fetal heart rate abnormalities, intrauterine growth retardation, cholestasis, or gestational diabetes) was collected.

Laboratory Assays

Immunologic assays were performed in a centralized laboratory (Sanofi Pasteur Global Clinical Immunology Laboratory, Swiftwater, Pennsylvania). The antibody titer against the vaccine strain was measured in all samples by using the validated HI method described by the WHO Collaborating Center for Surveillance, Epidemiology, and CDC, Atlanta, Georgia (14). Serum samples were treated by an enzymatic treatment, heated to destroy nonspecific inhibitors, and adsorbed with turkey erythrocytes to avoid antispecies hemagglutinin binding. Hemagglutination was performed in a microtiter test by using turkey erythrocytes, with the A/California/7/2009(H1N1v)-like reassortant strain used as the antigen. Serial 2-fold dilutions of the treated serum were used, with a starting dilution of 1:10. The sample titer was the highest dilution that inhibited hemagglutination completely. Negative samples were assigned a titer of 1:5. Hemagglutination inhibition was assayed in 2 different runs for each sample, and the geometric mean of the replicates were used for analyses.

We analyzed neutralizing activity by using an enzyme-linked immunosorbent assay read-out format microneutralization assay based on the methods described by the influenza reference laboratories of the CDC (14). The influenza microneutralization assay was validated according to International Conference on Harmonisation guidelines (15). Negative samples were assigned a titer of 1:5.

Statistical Analysis

According to the standard criteria for evaluating influenza vaccines (16), a sample size of 50 participants per group was needed. We planned to enroll 120 women (60 in each group) to obtain 50 informative participants per group. The immunologic efficacy and safety analyses presented here include all available data on vaccinated participants.

Immunogenicity was analyzed by using the standard HI end points (with 95% CIs) used by regulatory authorities for evaluating influenza vaccines (16–19). Seroprotection rate was defined as the percentage of women with an HI titer of 1:40 or greater. Seroconversion rate was defined as the percentage of women who had a prevaccination HI titer less than 1:10 and a postvaccination titer of 1:40 or greater or who showed a substantial antibody titer increase, defined as a prevaccination titer of 1:10 or greater and a 4-fold or greater increase in the postvaccination titer. Fold increase in geometric mean titer (GMT) was defined as the geometric mean of the ratio of the antibody titer after vaccination to the antibody titer on day 0.

Exact 95% binomial CIs were calculated for proportions. For GMT and mean fold increase in GMT, the mean and 95% CI were calculated for the $\log_{10}$-transformed titers and then transformed back to the original units by exponentiation (20). The Spearman correlation coefficient was calculated between HI titers and neutralizing antibody titers.

Univariate analyses were performed to investigate factors that may be associated with maternal seroprotection before and after vaccination: age, twin pregnancies, seasonal influenza vaccination in the past 3 years, gestation group, and site. We used the Kruskal–Wallis test for site and the Wilcoxon rank-sum test for all other variables. The end points analyzed were GMT and fold increase in GMT. The analysis of GMTs was a post hoc decision because the end points specified in the protocol (seroprotection rate and seroconversion rate) could not be used with rates greater than 90%.

For cord serum data in twin births, 1 of the infants was randomly selected. We performed a Wilcoxon signed-rank test to compare cord serum and maternal titers and calculated the Spearman correlation coefficient between these titers at delivery. All analyses were performed with SAS, version 9.1 (SAS Institute, Cary, North Carolina).

Role of the Funding Source

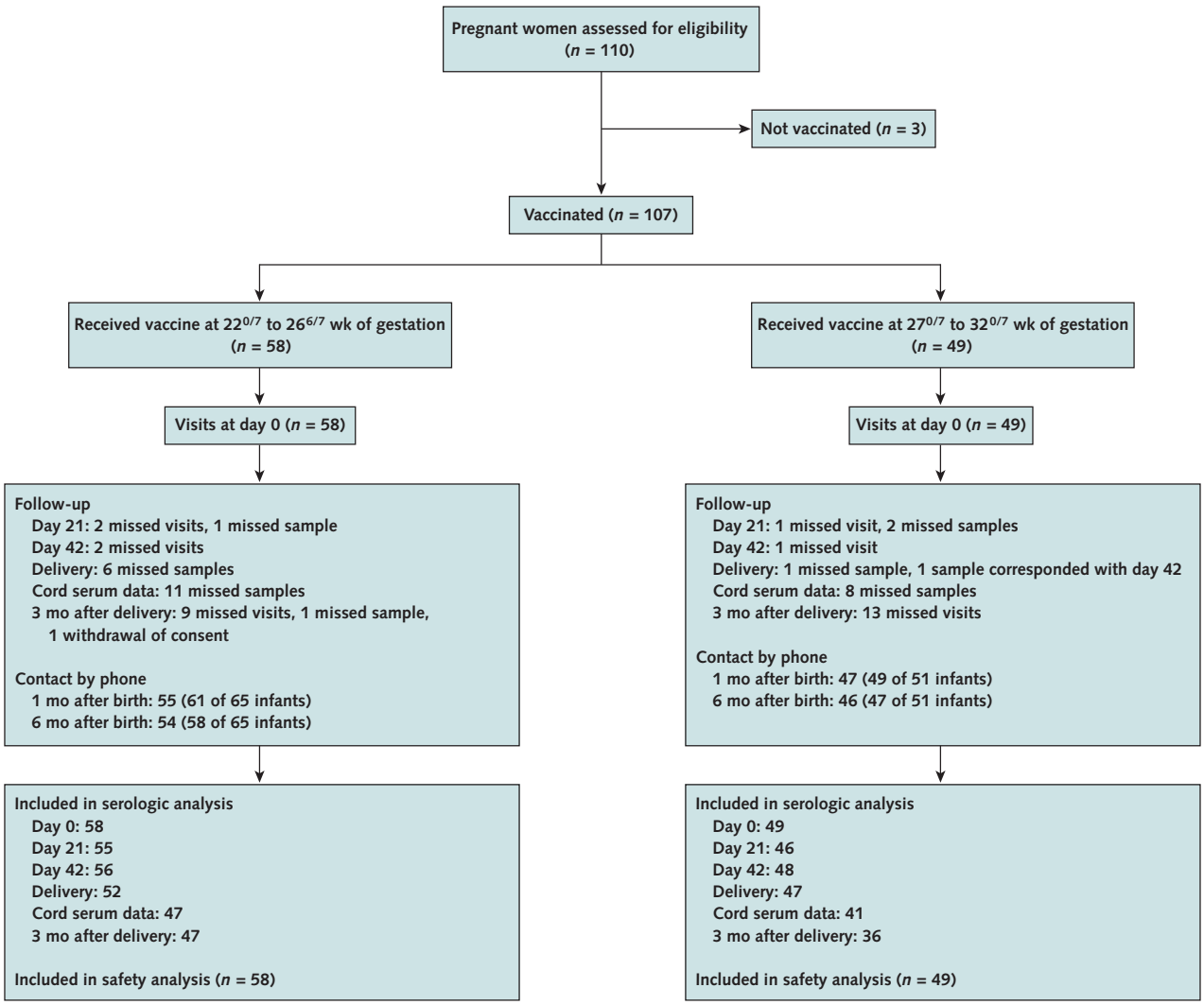
Our trial was funded by the French National Institute of Health and Medical Research. Sanofi-Pasteur provided the vaccines and performed immunologic tests. The funding sources had no role in the design, conduct, or analysis of our study or in the decision to submit the manuscript for publication.

RESULTS

Study Population

We included 110 pregnant women from 3 November to 4 December 2009, of whom 107 were vaccinated (**Figure**). Overall, 58 women in the $22^{0/7}$ to $26^{6/7}$ weeks of gestation group and 49 in the $27^{0/7}$ to $32^{0/7}$ weeks of

Figure. **Study flow diagram.**



gestation group were vaccinated and included in the analysis (**Figure**). **Table 1** lists the demographic and clinical characteristics of the groups. Nine of the pregnancies resulted in twin births. The women gave birth to 116 neonates. Median gestational age at delivery was 39 weeks (interquartile range [IQR], 38 to 40 weeks). Preterm delivery occurred in 9 women, 5 of whom had twins. Median infant birthweight was 3160 g (IQR, 2903 to 3570 g); 12 infants had a birthweight less than 2500 g (8 from 6 twin pregnancies and 4 from singleton pregnancies). The median gestational age of these infants at delivery was 36.2 weeks (IQR, 35.5 to 36.4 weeks) compared with 39.5 weeks (IQR, 38.6 to 40.4 weeks) for the 97 others.

Immunogenicity

Hemagglutination Inhibition

At baseline, 19% (95% CI, 12% to 27%) of women had HI antibodies against the A/California/7/2009(H1N1v) strain with titers of 1:40 or greater (**Table 2**). The only factor associated with maternal seroprotection before vaccination was vaccination for seasonal influenza in the past 3 years (GMT, 22.4 [CI, 11.7 to 43.2]) for vaccinated women vs. 9.2 [CI, 7.2 to 11.7] for unvaccinated women; $P = 0.001$). The following variables were not associated with maternal HI GMT before vaccination: age (GMT, 11.0 [CI, 8.0 to 15.0] for women aged <35 years vs. 10.9 [CI, 7.5 to 15.9] for those aged ≥35 years; $P = 0.72$), twin pregnancies (GMT, 8.9 [CI, 2.6 to 30.6] vs. 11.2 [CI, 8.8 to 14.2] for those with a singleton pregnancy; $P = 0.23$), gestational age group (GMT, 11.1 [CI, 8.0 to 15.5] for the $22^{0/7}$- to $26^{6/7}$-week group vs. 10.7 [CI, 7.5 to 15.4] for the $27^{0/7}$- to $32^{0/7}$-week group; $P = 0.74$), or site (GMT for women in site 1, 8.3 [CI, 5.4 to 12.8]; site 2, 19.6 [CI, 8.0 to 47.7]; site 3, 10.5 [CI, 6.8 to 16.4]; site 4, 9.4 [CI, 6.1 to 14.3]; and site 5, 11.7 [CI, 5.4 to 25.6]; $P = 0.58$).

At day 21 after vaccination, 98% (CI, 93% to 100%) of the women had HI antibody titers of 1:40 or greater, with a seroconversion rate of 93% (CI, 86% to 97%) and a fold increase in GMT of 67.4 (CI, 49.3 to 92.1). Women with a previous vaccination for seasonal influenza in the past 3 years had a significantly lower fold increase in GMT (30.8 [CI, 13.9 to 68.0] vs. 79.9 [CI, 57.0 to 111.8] for nonvaccinated women; $P = 0.023$). A lower maternal immune response was observed in women with twin pregnancies (GMT, 179.6 [CI, 55.0 to 586.8] vs. 796.3 [CI, 609.9 to 1039.6] for women with a singleton pregnancy; $P = 0.006$). The following variables were not associated with maternal HI GMT at day 21: age (GMT, 747.4 [CI, 526.1 to 1061.8] for women aged <35 years vs. 608.2 [CI, 395.45 to 935.4] for those ≥35 years; $P = 0.29$); seasonal influenza vaccination in the past 3 years (GMT, 604.1 [CI, 341.3 to 1069.1] vs. 719.4 [CI, 527.7 to 980.7] for nonvaccinated women; $P = 0.47$); gestational age group (GMT, 753.9 [CI, 522.0 to 1089.0] for women in the $22^{0/7}$- to $26^{6/7}$-week group vs. 635.2 [CI, 420.0 to 960.6] for those in the $27^{0/7}$- to $32^{0/7}$-week group; $P = 0.67$); and site (GMT for women in site 1, 1066.6 [CI, 515.7 to 2206.1]; site 2, 675.1 [CI, 294.9 to 1545.4]; site 3, 462.5 [CI, 287.3 to 744.4]; site 4, 835.5 [CI, 473.1 to 1475.5]; and site 5, 749.2 [CI, 368.7 to 1522.3]; $P = 0.23$).

Hemagglutination inhibition antibody titers of 1:40 or greater were observed in 98% of the women (CI, 93% to 100%) at day 42, 92% (CI, 85% to 96%) at delivery, and 90% (CI, 82% to 96%) at 3 months after delivery (**Table 2**). At delivery, twin pregnancies were significantly associated with lower maternal antibody titers (GMT, 67.3 [CI, 10.0 to 453.1] vs. 301.5 [CI, 229.3 to 396.4] for women with a singleton pregnancy; $P = 0.037$).

Titers of 1:40 or greater were observed in 95% (CI, 89% to 99%) of the 88 cord serum samples, and GMTs were higher than maternal titers at delivery (413.4 [CI, 297.6 to 574.2] in cord serum samples vs. 275.3 [CI, 208.3 to 363.9] in mothers; mean paired cord serum–maternal difference, 393.6 [CI, 239.0 to 548.1]; $P < 0.001$) (**Table 2**). Maternal HI antibody titers at delivery were strongly correlated with neonatal HI titers ($R^2 = 0.86$; $P < 0.001$). In contrast, no relationship was found between neonatal seroprotection and gestational age at delivery or vaccination–delivery interval (data not shown).

Microneutralization Assay

Neutralizing antibody titers were strongly correlated with HI antibody titers ($R^2 = 0.96$; $P < 0.001$). At baseline, 30% (CI, 21% to 40%) of the women had neutralizing antibodies against the A/California/7/2009(H1N1v) strain with titers of 1:40 or greater. At days 21 and 42 after vaccination and at delivery, the respective proportions of women with titers of 1:40 or greater were 100% (CI, 96% to 100%), 100% (CI, 97% to 100%), and 99% (CI, 95% to 100%). At day 21, the seroconversion rate was 96% (CI, 90% to 99%). Among cord serum samples, 98% had titers of 1:40 or greater (CI, 92% to 100%) and the GMTs were higher than maternal titers at delivery (1909.1 [CI, 1341.7 to 2716.5] vs. 1433.9 [CI, 1050.2 to 1957.9]; mean paired cord serum–maternal difference, 1260.3 [CI, 803.0 to 1717.7]; $P < 0.001$). Three months after delivery, the pro-

Table 1. **Participant Characteristics**

Characteristic	$22^{0/7}$ to $26^{6/7}$ Weeks of Gestation	$27^{0/7}$ to $32^{0/7}$ Weeks of Gestation	Total
Women			
Total, *n*	58	49	107
Median age (IQR), *y*	31.9 (30.2–36.4)	32.2 (30.0–36.4)	32.0 (30.1–36.4)
Age >35 y, *n (%)*	21 (36)	19 (39)	40 (37)
Received seasonal influenza vaccine in the past 3 y, *n (%)*	11 (19)	10 (20)	21 (20)
Median interval between vaccination and delivery (IQR), *wk*	16 (14–17)	10 (8–11)	12 (10–16)
Delivery <37 wk, *n (%)*	7 (12)*	2 (4)†	9 (8)
Cesarean delivery, *n (%)*	11 (19)	8 (16)	19 (18)
Twin pregnancy, *n (%)*	7 (12)	2 (4)	9 (8)
Infants			
Total, *n*	65	51	116
Birthweight, *g*			
Median (IQR)	3130 (2825–3570)	3180 (2965–3570)	3160 (2903–3570)
Range	1510–4310	1890–4440	1510–4440
Birthweight <2500 g, *n (%)*	11 (17)	1 (2)	12 (10)
Singleton pregnancies, *n/N (%)*	4/51 (8)	0/47 (0)	4/98 (4)
Twin pregnancies, *n/N (%)*	7/14 (50)	1/4 (25)	8/18 (44)
Median gestational age at vaccination (IQR), *wk*	23.0 (22.0–25.0)	30.0 (28.0–31.0)	26.0 (23.0–29.0)
Gestational age at delivery, *wk*			
Median (IQR)	40 (38–41)	39 (38–40)	39 (38–40)
Range	33–42	36–42	33–42

IQR = interquartile range.
* Three of the women gave birth to twins.
† Both women gave birth to twins.

Table 2. **Immunogenicity and Persistence of Immune Response of 1 Dose of Vaccine Administered to Pregnant Women***

Variable	$22^{0/7}$ to $26^{6/7}$ Weeks of Gestation	$27^{0/7}$ to $32^{0/7}$ Weeks of Gestation	All Participants
Prevaccination at day 0			
Women, *n*	58	49	107
Geometric mean titer (95% CI)	11.1 (8.0–15.5)	10.7 (7.5–15.4)	11.0 (8.6–13.9)
Seroprotection rate†			
Actual rate, *n (%)*	11 (19)	9 (18)	20 (19)
95% CI‡	10–31	9–32	12–27
Postvaccination at day 21			
Women, *n*	55	46	101
Geometric mean titer (95% CI)	753.9 (522.0–1089.0)	635.2 (420.0–960.6)	697.3 (532.0–914.1)
Seroprotection rate†			
Actual rate, *n (%)*	54 (98)	45 (98)	99 (98)
95% CI‡	90–100	88–100	93–100
Seroconversion rate§			
Actual rate, *n (%)*	52 (95)	42 (91)	94 (93)
95% CI§	85–99	79–98	86–97
Fold increase in geometric mean titer (95% CI)‖	64.8 (42.9–97.8)	70.6 (43.0–115.9)	67.4 (49.3–92.1)
Postvaccination at day 42			
Women, *n*	56	48	104
Geometric mean titer (95% CI)	496.6 (345.9–712.9)	397.4 (267.3–590.7)	448.0 (344.2–583.2)
Seroprotection rate†			
Actual rate, *n (%)*	55 (98)	47 (98)	102 (98)
95% CI‡	90–100	89–100	93–100
At delivery			
Women, *n*	52	47	99
Geometric mean titer (95% CI)	270.9 (180.6–406.4)	280.2 (188.7–416.2)	275.3 (208.3–363.9)
Seroprotection rate†			
Actual rate, *n (%)*	48 (92)	43 (91)	91 (92)
95% CI‡	81–98	80–98	85–96
3 mo after delivery			
Women, *n*	47	36	83
Geometric mean titer (95% CI)	280.2 (186.5–421.1)	261.4 (160.1–427.0)	271.9 (200.0–369.7)
Seroprotection rate*			
Actual rate, *n (%)*	44 (94)	31 (86)	75 (90)
95% CI‡	82–99	71–95	82–96
In neonates			
Cord serum samples, *n*	47	41	88
Geometric mean titer (95% CI)	387.6 (246.1–610.7)	445.0 (271.0–730.6)	413.4 (297.6–574.2)
Seroprotection rate†			
Actual rate, *n (%)*	45 (96)	39 (95)	84 (95)
95% CI‡	85–99	83–99	89–99
Median ratio of neonate–mother HI antibody titers (IQR)	1.4 (1.4–2.0)¶	2.0 (1.0–2.8)	1.4 (1.0–2.8)**

HI = hemagglutination inhibition; IQR = interquartile range.
* Immunogenicity measured at day 21 after vaccination and persistence measured at day 42, delivery, and 3 mo after delivery.
† Proportion of participants with a postvaccination HI antibody titer ≥1:40.
‡ 95% CI of the estimated percentage in the total population.
§ Proportion of participants with a prevaccination HI antibody titer <1:10 and a postvaccination titer ≥1:40 or who showed a substantial increase in antibody titer (prevaccination titer ≥1:10 and ≥4-fold increase in postvaccination titer).
‖ Geometric mean of the ratio of the antibody titer after vaccination to the antibody titer on day 0.
¶ Calculated by using only 46 of the 47 samples.
** Calculated by using only 87 of the 88 samples.

portion of women with titers of 1:40 or greater was 89% (CI, 80% to 95%).

Adverse Events

Serious adverse events were reported for 13 women (7 cases of threatened preterm labor; 3 cases of fetal growth restriction; and 1 case each of preterm rupture of membranes, fetal heart rate abnormality, renal colic, fall with abdominal trauma, gestational cholestasis and gestational diabetes, preeclampsia, and bladder calculus). Serious adverse events were reported in 7 neonates (2 inguinal hernias and 1 case each of familial glucocorticoid deficiency, Robertsonian translocation, thoracic cystic lymphangioma, patent ductus arteriosus, supraventricular tachycardia with cardiac insufficiency, and cryptorchidism). The independent adjudication committee considered none of these events to be related to the vaccine. At least 1 specified local adverse event was reported for 22 women (21%); pain was most common (**Table 3**). At least 1 systemic adverse event was reported by 33 women (31%); asthenia and headache were most common (**Table 3**). Most specified adverse

Table 3. **Local and General Adverse Events Considered Related to the Influenza A(H1N1v) Vaccine**

Event	$22^{0/7}$ to $26^{6/7}$ Weeks of Gestation (n = 58)	$27^{0/7}$ to $32^{0/7}$ Weeks of Gestation (n = 49)	All Participants (n = 107)
Women with ≥1 reaction, *n (%)*			
Injection-site reaction	15 (26)	7 (14)	22 (21)
Systemic reaction	19 (33)	14 (29)	33 (31)
Injection-site or systemic reaction	27 (47)	18 (37)	45 (42)
Injection-site reactions, *n (%)*			
Pain	14 (24)	6 (12)	20 (19)
Induration	2 (4)	1 (2)	3 (3)*
Erythema	1 (3)	1 (2)	2 (2)†
Systemic reactions, *n (%)*			
Asthenia	12 (21)	12 (24)	24 (22)
Headache	6 (10)	4 (8)	10 (9)
Myalgia	2 (3)	1 (2)	3 (3)
Arthralgia	0	2 (4)	2 (2)
Hyperhidrosis	0	2 (4)	2 (2)
Pyrexia	1 (2)	0	1 (1)
Chills	2 (3)	0	2 (2)

* Diameters of 1.5 and 8 cm (the diameter of the third case is missing).
† Diameters of 0.5 and 3 cm.

events were mild (61%) to moderate (29%) in intensity. No adverse event of special interest was reported.

No confirmed influenza episode occurred during the study period. According to the mothers' answers to the questionnaire given at 1 and 6 months after birth, 28 infants (27%) had fever associated with another respiratory symptom during this period. Ten reported an influenza infection in a member of their household. None of the 28 infants was hospitalized for clinically diagnosed influenza, so no subsequent laboratory exploration was performed.

DISCUSSION

To our knowledge, ours is the first published trial to evaluate both maternal immune response and neonatal seroprotection from a single dose of a pandemic vaccine in pregnant women. Ninety-eight percent of the women who received a single 15-mcg dose of nonadjuvanted vaccine achieved an HI antibody titer of 1:40 or greater. The intervention was also associated with a 93% rate of seroconversion and a fold increase in GMT of HI antibodies of 67.4 on day 21 after vaccination. These results are consistent with the regulatory requirements for the use of vaccine in adults issued by the European Union Committee for Medicinal Products for Human Use and the U.S. Center for Biologics Evaluation and Research (17–19). These results show amplitude of the HI response similar to that observed at the same time point in other trials that investigated a nonadjuvanted influenza A(H1N1v) vaccine in healthy nonpregnant participants. After injection of a single 15-mcg dose, 100% (CI, 98% to 100%) of healthy adults aged 18 to 64 years achieved vaccine-homologous HI antibody titers of 1:40 or greater (21).

The choice of the cutoff that defines seroprotection may seem controversial. In Europe, regulatory authorities use different criteria to assess the immunogenicity of influenza vaccine, including the proportion of participants with an HI antibody titer greater than 1:40. This cutoff is based on challenge efficacy studies performed in the 1970s (22). However, because the HI assay is not standardized across laboratories, the relevance of these data can be questioned. Nevertheless, a recent analysis of published data (23) indicates that 70% of participants are protected at a titer of 1:40, with protection increasing gradually with higher titers. In the absence of a widely accepted immune correlate of protection for influenza, the analysis of HI seroprotection rates is generally considered a useful indicator of protection in vaccinated persons.

Of note, the proportion of women with an HI antibody titer of 1:40 or greater remained high at both delivery and 3 months after delivery, which may contribute to protecting infants from influenza exposure. Although we originally planned to investigate the protection conferred by 2 doses of vaccine, we could not do so because the French National Authority for Health recommended using a single dose shortly before the start of our trial. In the context of safety concerns about pandemic H1N1 vaccines, the use of 2 doses was considered unacceptable. We also decided to enroll patients over 2 gestational age periods to investigate differences in immune response and neonatal seroprotection by gestational age at vaccination and by interval between vaccination and delivery.

The high proportion of women already seroprotected at baseline (19%) may be the result of cross-immunoreactivity with previous seasonal influenza vaccination in the past 3 years. In our sample, 20% of participants reported receiving seasonal influenza vaccine in the past 3 years. A high association was found between seroprotection at baseline and previous seasonal vaccination. However, previous subclinical H1N1 infection may also be an explanation. In France, the first wave of H1N1 infection was reported in July 2009.

Our findings are supported by the preliminary results of a trial in pregnant women by the U.S. National Institute of Allergy and Infectious Diseases (24). The nonadjuvanted influenza A(H1N1v) vaccine used in this clinical trial (also manufactured by Sanofi Pasteur) was administered to pregnant women in their second or third trimester of pregnancy. After a single dose, a titer of 1:40 or greater was observed in 23 of 25 women (92%) who received 15 mcg and in 24 of 25 women (96%) who received 30 mcg. Ohfuji and colleagues (25) also recently reported strong maternal immune response after H1N1 vaccination (25).

Human fetuses obtain maternal IgG antibodies by placental transfer. These antibodies protect neonates from infectious diseases during the first months of life, when their immune system is not yet fully developed and functioning (26). Immunoglobulins are large hydrophilic molecules (150 kDa) that cannot cross the placental barrier by simple diffusion and require active transport, which involves neonatal Fc receptor. The syncytiotrophoblast expresses this receptor and internalizes maternal IgG. The vesicle fuses thereafter with the membrane on the fetal side of the syncytiotrophoblast and releases IgG into the fetal capillaries (27, 28). Our study showed that a single 15-mcg dose of nonadjuvanted vaccine in pregnant women conferred a high rate of seroprotection (HI antibody titer $\geq$1:40) in 95% of the neonates. The transplacental transfer of antibodies, defined as the GMT ratio of the mean HI antibody titer in infants and mothers, was greater than 1, which suggests that the placenta has an active role in maternofetal transfer of immunoglobulin. In contrast, a study that used pandemic MF59-adjuvanted 2009 H1N1 vaccine (29) found a transplacental transfer of antibody of only 0.55 at delivery; however, in addition to using an adjuvanted vaccine, the investigators obtained venous blood instead of cord blood in the 2 days after delivery, which might account for the difference. Another study of seasonal trivalent influenza immunization during pregnancy (30) reported similar transplacental transfer of antibody (0.8 to 1.1), using samples of cord serum blood at delivery.

Safety is a major concern when recommending vaccination, especially for pregnant women. Because the 2009 H1N1v monovalent vaccine was manufactured by the same process as the seasonal influenza vaccine, similar safety and efficacy in pregnant women were expected. During the 2009 H1N1 vaccination campaign, the French Network of Pharmacovigilance, which records spontaneous notifications of adverse events, found no worrying safety reports in pregnant women and infants (31). In our study, the nonadjuvanted 2009 H1N1 vaccine with 15 mcg of hemagglutinin had an acceptable tolerance profile after a single dose in pregnant women. Adverse maternal events were mainly mild to moderate in severity.

Our study has limitations. First, it was not powered to detect low-frequency events. Our study had only 80% power to detect adverse events with an incidence less than 1.5%. Second, at this early stage of using the vaccine in pregnant women, our sample was restricted to healthy pregnant women and excluded those with comorbid conditions, such as cardiac disease, chronic liver disease, or diabetes before pregnancy; those with a history of premature delivery or eclampsia; or those whose fetus had morphologic abnormalities. Similarly, women were included after ultrasonography was done between $21^{0/7}$ and $23^{0/7}$ weeks of gestation, to avoid including cases of fetal malformation. Standard contraindications to vaccination were also applied in our trial; thus, the excellent immune response after 1 dose of H1N1 vaccine may not be generalizable to patients with diseases known to impair immune response. Finally, HI antibody titers are reported only at birth for infants (from cord serum). We chose not to obtain blood samples from infants to improve the feasibility of the study during this pandemic period. Although no influenza episode was reported in the infants in our study up to 6 months after birth, we have no long-term immunization data for the infants. However, the high level of seroprotection and GMT in the infants at birth suggests that seroprotection could persist for months. Zuccotti and colleagues (29) observed HI antibody titers greater than 1:40 in 95.6% of infants at birth, 95.6% at 2 months, and 81.2% at 5 months. Therefore, vaccination during pregnancy protects infants during the first months of life, when vaccination cannot be performed. After 6 months, infants can be immunized against influenza.

In conclusion, a single dose of a nonadjuvanted influenza A(H1N1v) vaccine with 15 mcg of hemagglutinin induces a strong immune response in pregnant women in their second and third trimester and a high rate of seroprotection in neonates.

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Appendix Figure. **Questionnaire for child follow-up.**

QUESTIONNAIRE OF CHILD FOLLOW-UP*

* In case of multiple pregnancy, complete one questionnaire for each child

Identification study number of patient: ☐☐☐ Center # ☐☐☐ Order # ☐☐☐☐ Anonymous code

Date of contact with patient: ☐☐ ☐☐ ☐☐☐☐ **M1** ☐ **M6** ☐

➢ **Since the release of maternity ward, did your child develop symptoms such as:**

- Fever ..... Yes ☐ No ☐
  If yes, highest temperature: ☐☐,☐ °C Duration: ☐☐ days
- Cough ..... Yes ☐ No ☐
- Rhinorrhea (runny nose) ..... Yes ☐ No ☐
- Nasal obstruction (stuffy nose) ..... Yes ☐ No ☐
- Difficulty in breathing ..... Yes ☐ No ☐

➢ **Did a doctor examine your child for these symptoms?** ..... Yes ☐ No ☐
If yes, what was the diagnosis? .....
If influenza, was a nasal swab done? ..... Yes ☐ No ☐
If yes, was the diagnosis of influenza A/H1N1 confirmed? ..... Yes ☐ No ☐

➢ **Was your child hospitalized?** ..... Yes ☐ No ☐
If yes, reason: .....
Was influenza A/H1N1 diagnosed? ..... Yes ☐ No ☐

➢ **Since the release of maternity ward, has a close contact of the child got influenza?** ..... Yes ☐ No ☐
If yes, specify: ○ Mother ○ Father ○ Brother ○ Sister
○ Other, specify: .....

➢ Did you breastfeed your child? ..... Yes ☐ No ☐
If yes, on-going breastfeeding ..... Yes ☐ No ☐
If no, date of breastfeeding discontinuation: ☐☐ ☐☐ ☐☐☐☐

For investigation center: This questionnaire will be completed with the patient at the postnatal visit at M1 and by phone at M6 (phone contact is possible at M1)

Thank you for faxing this questionnaire to SC10