

However, few studies have examined the immunogenicity and reactogenicity of the influenza vaccine administered to pregnant women. This lack of scientific evidence might contribute to the low level of vaccine coverage among pregnant women. The annual influenza vaccination has been recommended to pregnant women in the United States for more than a decade, but vaccine coverage has remained at a low level, compared with that of other high-risk groups [11]. Pregnant women may be reluctant to receive the vaccination because they are concerned about the effect of any medications (including vaccines) on their fetuses. However, 1 report has found that the attitude of health care providers also contributes to the lack of vaccine coverage among pregnant women. According to this report, 30% of health care providers did not believe in the safety and effectiveness of influenza vaccine among pregnant women, and 60% of health care providers did not know that pregnant women were at high risk for influenza-associated complications [12]. To achieve a high rate of influenza vaccination among pregnant women, it is essential to accumulate evidence about the influenza disease burden and the immunogenicity, reactogenicity, and effectiveness of vaccination among this group.

Studies of the 2009 pandemic influenza vaccine found that a single dose of vaccine with 15  $\mu$ g of antigen induced sufficient immune responses among adults [13–16]. However, most of these studies excluded pregnant women from the immunogenicity analyses. To provide some information in a national decision about the number of doses of a monovalent 2009 influenza A (H1N1) vaccine to recommend for pregnant women, the present study examined the immunogenicity of 2 doses of vaccine among pregnant women in Japan. When researchers are evaluating the antibody induction by a vaccine, the effect of potential predictors, such as age and prevaccination titer, should be considered [17]. Thus, the induction of serum hemagglutination inhibition (HAI) antibody was assessed by 3 conventional parameters—the fold rise, the seroresponse rate ( $\geq 4$ -fold rise), and the seroprotection rate (postvaccination titer  $\geq 1:40$ )—and the independent effects of potential predictors for antibody induction were then evaluated.

## METHODS

### Study Subjects

The study subjects were pregnant women recruited from 2 medical institutions of obstetrics in Osaka, Japan. All subjects provided written informed consent after the nature and possible consequences of the study had been explained. The study protocol was approved by the ethics committee at the Osaka City University Graduate School of Medicine and was performed in accordance with the Declaration of Helsinki. None of the applicants met the exclusion criteria for eligibility, including a history of 2009 influenza A (H1N1) infection, an acute febrile illness or signs of severe acute illness at the time of

vaccination, a history of anaphylaxis due to vaccine components, or other inappropriate condition to receive vaccination. A total of 150 pregnant women were enrolled. The subjects received 2 subcutaneous injections of the 2009 monovalent inactivated influenza A (H1N1) vaccine into their arms 3 weeks apart (Lot. NM001A; Kitasato). Each vaccine contained 15  $\mu$ g of hemagglutinin antigen. The vaccines did not contain thimerosal. The seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A (New York Medical College), distributed by the Centers for Disease Control and Prevention in the United States. The vaccine was prepared in embryonated chicken eggs by using standard methods for the production of seasonal trivalent inactivated vaccine.

### Information Collection

At the time of recruitment, subjects completed a self-administered questionnaire to collect the following information: age at vaccination, height and body weight before pregnancy, underlying illnesses (ie, heart disease, renal disease, liver disease, atopy, or asthma), 2009–2010 seasonal influenza vaccination before recruitment, and the date of vaccination (if vaccinated). The 2009–2010 seasonal influenza vaccine strains were A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), and B/Brisbane/60/2008. Additionally, the subjects' obstetricians completed a structured questionnaire to collect the following clinical information: gestational age, multiple pregnancy, and pregnancy-induced complications (ie, anemia, pregnancy-induced hypertension, or gestational diabetes).

### Measurement of Antibody Titer

Serum samples were collected at 3 time points: before vaccination (S0); 3 weeks after the first dose (S1); and 4 weeks after the second dose (S2). All serum specimens were kept at  $-80^{\circ}\text{C}$  until assayed at the same time. Serum antibody levels to hemagglutinin were measured by the standard microtiter HAI method [18] with the same antigens as in the vaccine. All samples were assayed at the Kitasato Institute in February 2010.

### Statistical Analyses

The following outcomes were calculated to assess the immunogenicity of influenza vaccine: the geometric mean titer; the fold rise; the seroresponse rate ( $\geq 4$ -fold rise); and the seroprotection rate (postvaccination titer  $\geq 1:40$ ). For data processing, titers  $< 1:10$  were regarded as 1:5, and reciprocal antibody titers were analyzed after logarithmic transformation. The results were presented in the original scale by calculating the antilogarithm. Stratified analyses were performed to examine the effect of the following potential confounders: body mass index before pregnancy (tertile or  $< 25.1$  and  $\geq 25.1$ ); trimester ( $< 16$ , 16–27, and  $\geq 28$  weeks); age at vaccination (tertile); 2009–2010 seasonal influenza vaccination (unvaccinated and vaccinated); duration between seasonal vaccination and H1N1

vaccination (unvaccinated,  $\geq 20$  days, and  $\leq 19$  days) and prevaccination titer ( $<1:10$ ,  $1:10$ – $1:20$ , and  $\geq 1:40$ ). The significance of fold rise within a category was assessed by the Wilcoxon signed-rank test, and intercategory comparisons were made by either the Wilcoxon rank-sum test or the Kruskal-Wallis test. The  $t$  test,  $\chi^2$  test, or Mantel-extension method for the trend test were also used where appropriate. Furthermore, the independent effects of potential confounders on antibody induction were evaluated by logistic regression. The models were constructed with seroresponse or seroprotection as the dependent variable and the above-mentioned potential confounders as explanatory variables. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. All tests were 2-sided. All analyses were performed using SAS, version 9.1.3 (SAS Institute).

## RESULTS

A total of 150 pregnant women received the first dose of vaccine between 7 November and 27 November 2009, and serum samples at 3 weeks after the first dose were collected from all of the subjects. Among these women, 142 received the second dose between 3 December and 18 December 2009, and serum samples at 4 weeks after the second dose were collected from 137 subjects. All subjects had a singleton pregnancy. Only 1 subject experienced a confirmed influenza A virus infection (as determined by the rapid test) between the first and the second dose and thus was excluded from the analyses. Eventually, data from 149 pregnant women were used for the immunogenicity analyses of the first dose, and data from 137 pregnant women were used for the analyses of the second dose. None of the subjects received both the 2009–2010 seasonal influenza vaccine and the 2009 monovalent influenza A (H1N1) vaccine at the same time. No severe adverse events for the pregnant women or their fetuses occurred after the first or second dose.

The subject characteristics are shown in Table 1. The mean age was 30.6 years, and half of the women were in the third trimester. Only a small number of subjects had pregnancy-induced complications, such as anemia (3%) or hypertension (1%). A total of 23% of the subjects received the 2009–2010 seasonal influenza vaccine before the H1N1 vaccination (Table 1).

The results of the antibody response are summarized in Table 2. The first dose of vaccine induced an average increase in the HAI antibody level of  $\geq 10$ -fold ( $P < .001$ ). The seroresponse rate was 91% (95% CI, 86%–96%), and the seroprotection rate was 89% (95% CI, 84%–94%). According to conventionally used international criteria [19, 20], the seroconversion rate was at the same level as the seroresponse rate (91%; 95% CI, 86%–96%). The second vaccination conferred little additional induction of antibodies.

The parameters of immunity (ie, fold rise, seroresponse rate, and seroprotection rate) were similar irrespective of body

**Table 1. Characteristics in Pregnant Women**

Characteristics	Study subjects (N = 149)
<b>Body mass index before pregnancy</b>	
Mean (SD)	20.7 (2.5)
Median (range)	20.1 (16.9–30.8)
<b>Age at H1N1 vaccination, years</b>	
Mean (SD)	30.6 (5.4)
Median (range)	31.0 (17–41)
<b>Underlying illnesses before pregnancy</b>	
Heart disease	1 (1)
Liver disease	1 (1)
Atopic dermatitis	8 (5)
Drug allergy	7 (5)
Food allergy	24 (16)
<b>Gestational age, weeks</b>	
First trimester (<16)	26 (17)
Second trimester (16–27)	46 (31)
Third trimester (28+)	77 (52)
<b>Pregnancy-induced complications</b>	
Pregnancy-induced hypertension	
Present	1 (1)
Unknown	3
Anemia	
Present	4 (3)
Unknown	3
Gestational diabetes	
Present	0 (0)
Unknown	4
2009–2010 seasonal influenza vaccination received	35 (23)

**NOTE.** Data are expressed as no. (%) of women, unless otherwise indicated.

mass index before pregnancy, trimester, or age at vaccination (Table 2). However, women who had received the 2009–2010 seasonal influenza vaccine before the H1N1 vaccination had a smaller immune response. The women who received the seasonal vaccination and H1N1 vaccination within 19 days exhibited a lower fold rise, seroresponse rate, and seroprotection rate (after the first dose:  $P = .021$ ;  $P = .001$ ; and  $P < .001$  for each). On the other hand, when comparing the results across 3-tiered prevaccination titers, the mean fold rises were significantly lower among those with higher prevaccination titers (after the first dose,  $P < .001$ ). Subjects with higher prevaccination titers also had lower seroresponse rates but higher seroprotection rates with clear dose-response relationships (after the first dose,  $P < .001$  and  $P = .052$ ).

Even after considering the effect of potential confounders, the group who had received the 2009–2010 seasonal influenza vaccine, especially within a short period ( $\leq 19$  days) between seasonal vaccination and H1N1 vaccination, had a decreased seroresponse after the first dose of H1N1 vaccine (Table 3). The adjusted OR (95% CI) of the vaccinated group was 0.15 (0.03–

**Table 2. Immunoresponses to Monovalent 2009 Influenza A (H1N1) Vaccine Among Pregnant Women**

Category	N	Geometric mean <sup>a</sup>			Fold rise <sup>a</sup>		After first vaccination <sup>b</sup>		After second vaccination <sup>bc</sup>		
		Before vaccination (S0)	After first vaccination (S1)	After second vaccination (S2) <sup>c</sup>	S1/S0	S2/S0 <sup>c</sup>	≥4-fold rise No. (%)	≥1:40 No. (%)	≥4-fold rise No. (%)	≥1:40 No. (%)	
Entire sample	149	8	139	114	17.1	14.1	136 (91)	132 (89)	123 (87)	124 (91)	
Body mass index before pregnancy											
<19.2	50	9	147	116	16.2 ( <i>P</i> < .001)	12.9 ( <i>P</i> < .001)	46 (92)	45 (90)	39 (87)	41 (91)	
19.2–21.4	49	7	111	92	16.7 ( <i>P</i> < .001)	14.1 ( <i>P</i> < .001)	45 (92)	40 (82)	40 (91)	37 (84)	
≥21.5	50	9	164	137	18.4 ( <i>P</i> < .001)	15.3 ( <i>P</i> < .001)	45 (90)	47 (94)	44 (92)	46 (96)	
			( <i>P</i> = .06)	( <i>P</i> = .30)	( <i>P</i> = .35)	( <i>P</i> = .88)	( <i>P</i> = .91)	( <i>P</i> = .72)	( <i>P</i> = .53)	( <i>P</i> = .41)	( <i>P</i> = .40)
<25.1	140	8	133	113	16.3 ( <i>P</i> < .001)	14.0 ( <i>P</i> < .001)	127 (91)	123 (88)	114 (89)	115 (90)	
≥25.1	9	9	296	137	31.6 ( <i>P</i> = .004)	16.0 ( <i>P</i> < .001)	9 (100)	9 (100)	9 (100)	9 (100)	
			( <i>P</i> = .97)	( <i>P</i> = .04)	( <i>P</i> = .55)	( <i>P</i> = .13)	( <i>P</i> = .82)	( <i>P</i> = 1.00)	( <i>P</i> = .60)	( <i>P</i> = .60)	( <i>P</i> = .60)
Trimester											
First	26	11	144	112	12.6 ( <i>P</i> < .001)	9.4 ( <i>P</i> < .001)	24 (92)	22 (85)	19 (76)	23 (92)	
Second	46	7	118	83	17.5 ( <i>P</i> < .001)	12.6 ( <i>P</i> < .001)	41 (89)	40 (87)	40 (91)	37 (84)	
Third	77	8	152	142	18.6 ( <i>P</i> < .001)	17.5 ( <i>P</i> < .001)	71 (92)	70 (91)	64 (94)	64 (94)	
			( <i>P</i> = .05)	( <i>P</i> = .63)	( <i>P</i> = .06)	( <i>P</i> = .49)	( <i>P</i> = .13)	( <i>P</i> = .86)	( <i>P</i> = .34)	( <i>P</i> = .02)	( <i>P</i> = .43)
Age at H1N1 vaccination (years)											
<29	46	8	136	126	17.5 ( <i>P</i> < .001)	16.8 ( <i>P</i> < .001)	42 (91)	41 (89)	37 (90)	37 (90)	
29–33	50	8	125	93	16.0 ( <i>P</i> < .001)	11.6 ( <i>P</i> < .001)	45 (90)	43 (86)	42 (89)	42 (89)	
≥34	53	9	158	128	17.8 ( <i>P</i> < .001)	14.7 ( <i>P</i> < .001)	49 (92)	48 (91)	44 (90)	45 (92)	
			( <i>P</i> = .87)	( <i>P</i> = .71)	( <i>P</i> = .28)	( <i>P</i> = .93)	( <i>P</i> = .39)	( <i>P</i> = .83)	( <i>P</i> = .80)	( <i>P</i> = .95)	( <i>P</i> = .79)
2009–2010 seasonal influenza vaccination											
Unvaccinated	114	8	159	127	20.3 ( <i>P</i> < .001)	16.1 ( <i>P</i> < .001)	108 (95)	105 (92)	102 (94)	101 (93)	
Vaccinated	35	9	90	74	9.8 ( <i>P</i> < .001)	8.4 ( <i>P</i> < .001)	28 (80)	27 (77)	21 (75)	23 (82)	
			( <i>P</i> = .41)	( <i>P</i> = .03)	( <i>P</i> = .07)	( <i>P</i> = .008)	( <i>P</i> = .028)	( <i>P</i> = .007)	( <i>P</i> = .02)	( <i>P</i> = .004)	( <i>P</i> = .09)
Duration between seasonal vaccination and H1N1 vaccination											
Unvaccinated	114	8	159	127	20.3 ( <i>P</i> < .001)	16.1 ( <i>P</i> < .001)	108 (95)	105 (92)	102 (94)	101 (93)	
≥20 days	17	8	120	101	15.4 ( <i>P</i> < .001)	13.3 ( <i>P</i> < .001)	17 (100)	15 (88)	14 (93)	13 (87)	
≤19 days	17	10	68	52	6.8 ( <i>P</i> < .001)	5.0 ( <i>P</i> = .002)	11 (65)	11 (65)	7 (54)	10 (77)	
			( <i>P</i> = .69)	( <i>P</i> = .08)	( <i>P</i> = .10)	( <i>P</i> = .021)	( <i>P</i> = .019)	( <i>P</i> = .001)	( <i>P</i> = .002)	( <i>P</i> < .001)	( <i>P</i> = .06)
Prevaccination titer											
<1:10	92	5	121	93	24.2 ( <i>P</i> < .001)	18.7 ( <i>P</i> < .001)	89 (97)	78 (85)	79 (93)	72 (85)	
1:10–1:20	46	13	173	152	13.0 ( <i>P</i> < .001)	11.7 ( <i>P</i> < .001)	43 (93)	43 (93)	40 (95)	42 (100)	
≥1:40	11	62	181	184	2.9 ( <i>P</i> = .008)	2.8 ( <i>P</i> = .016)	4 (36)	11 (100)	4 (40)	10 (100)	
			( <i>P</i> = .37)	( <i>P</i> = .07)	( <i>P</i> < .001)	( <i>P</i> < .001)	( <i>P</i> < .001)	( <i>P</i> = .05)	( <i>P</i> = .001)	( <i>P</i> = .007)	

**NOTE.** <sup>a</sup> Wilcoxon signed-rank test for intracategory comparisons, and either the Wilcoxon rank-sum test or the Kruskal-Wallis test for intercategory comparisons.

<sup>b</sup> Seroresponse rate (≥4-fold rise) and seroprotection rate (postvaccination titer ≥1:40).  $\chi^2$  test between 2 categories and the Mantel-extension method for trend test among 3 categories.

<sup>c</sup> The results of 137 study subjects who received second dose of vaccination and provided serum sample after second vaccination.

**Table 3. Association Between Selected Characteristics and Seroreponse Rate ( $\geq 4$ -Fold Rise) After First Dose of Vaccination**

Category	n/N (%)	Crude analysis			Multivariate model 1 <sup>a</sup>			Multivariate model 2 <sup>b</sup>		
		OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P
Body mass index before pregnancy										
<19.2	46/50 (92)	1.00			1.00			1.00		
19.2–21.4	45/49 (92)	0.98	(.23–4.15)	0.98	0.34	(.05–2.47)	0.28	0.53	(.06–4.55)	0.56
$\geq 21.5$	45/50 (90)	0.78	(.20–3.10)	0.73	0.42	(.06–3.14)	0.40	0.40	(.04–4.15)	0.44
		(Trend <i>P</i> = .72)			(Trend <i>P</i> = .38)			(Trend <i>P</i> = .41)		
Trimester										
First	24/26 (92)	1.00			1.00			1.00		
Second	41/46 (89)	0.68	(.12–3.80)	0.66	0.28	(.02–3.28)	0.31	0.30	(.02–5.30)	0.41
Third	71/77 (92)	0.99	(.19–5.22)	0.99	0.61	(.06–6.33)	0.68	0.39	(.03–5.72)	0.49
		(Trend <i>P</i> = .86)			(Trend <i>P</i> = .88)			(Trend <i>P</i> = .60)		
Age at H1N1 vaccination, years										
<29	42/46 (91)	1.00			1.00			1.00		
29–33	45/50 (90)	0.86	(.22–3.41)	0.83	0.95	(.17–5.44)	0.96	1.33	(.17–10.7)	0.79
$\geq 34$	49/53 (92)	1.17	(.28–4.95)	0.84	4.09	(.48–34.6)	0.20	5.82	(.45–75.5)	0.18
		(Trend <i>P</i> = .83)			(Trend <i>P</i> = .20)			(Trend <i>P</i> = .16)		
2009–2010 seasonal influenza vaccination										
Unvaccinated	108/114 (95)	1.00			1.00					
Vaccinated	28/35 (80)	0.22	(.07–.71)	0.01	0.15	(.03–.80)	0.03			
Duration between seasonal vaccination and H1N1 vaccination										
Unvaccinated	108/114 (95)	1.00						1.00		
$\geq 20$ days	17/17 (100)	Not applicable						Not applicable		
$\leq 19$ days	11/17 (65)	0.10	(.03–.37)	0.001				0.03	(.004–.29)	0.002
		(Trend <i>P</i> = .002)						(Trend <i>P</i> = .003)		
Prevaccination titer										
<1:10	89/92 (97)	1.00			1.00			1.00		
1:10–1:20	43/46 (93)	0.48	(.09–2.49)	0.39	0.27	(.04–1.86)	0.19	0.33	(.04–2.53)	0.29
$\geq 1:40$	4/11 (36)	0.02	(.004–.10)	<.001	0.01	(.00–.07)	<.001	0.01	(.00–.09)	<.001
		(Trend <i>P</i> < .001)			(Trend <i>P</i> < .001)			(Trend <i>P</i> = .001)		

**NOTE.** Logistic regression model. CI, confidence interval; OR, odds ratio.

<sup>a</sup> Model included body mass index before pregnancy, trimester, age at H1N1 vaccination, 2009–2010 seasonal influenza vaccination, and prevaccination titer.

<sup>b</sup> Model included body mass index before pregnancy, trimester, age at H1N1 vaccination, duration between seasonal and H1N1 vaccination, and prevaccination titer.

0.80). A higher prevaccination titer was also independently associated with a lower seroreponse (Trend *P* < .001).

As shown in Table 4, subjects with 2009–2010 seasonal influenza vaccination also had a statistically significant decrease in OR for seroprotection to the 2009 pandemic influenza A (H1N1) vaccine (OR, 0.24; 95% CI, 0.08–0.76). In contrast, subjects with higher prevaccination titers had increased ORs for seroprotection. There was no association between antibody responses and body mass index before pregnancy, trimester, and age at vaccination.

Additional analyses were conducted when the cut-off point of duration between seasonal influenza vaccination and 2009 pandemic influenza A (H1N1) vaccination was changed from 20 days to 14 days. Among 10 subjects with seasonal vaccination within 14 days, geometric mean titer levels at S0 and S1 were 10 and 49, respectively, which result in 4.9-fold rises after the first dose of H1N1 vaccination. The seroreponse rate was 60% and

the seroprotection rate was 50%. Multivariate analyses showed that ORs of subjects with seasonal vaccination within 14 days were lowered both for seroreponse and for seroprotection as outcome index (for seroreponse, OR, 0.03; 95% CI, 0.004–0.26; and for seroprotection, OR, 0.07; 95% CI, 0.01–0.35).

During the study periods, 6 subjects reported influenza-like illness (defined by acute febrile illness [temperature  $\geq 38.0^\circ\text{C}$ ] with 1 or more respiratory symptoms [nasal discharge or runny nose, sore throat, or cough]). However, even when these subjects were excluded from the analyses, the results were almost unchanged (data not shown).

## DISCUSSION

The present study shows that a single dose of 2009 influenza A (H1N1) vaccine induced sufficient immune responses among pregnant women irrespective of body mass index before

**Table 4. Association Between Selected Characteristics and Seroprotection Rate (Titer  $\geq$  1:40) After First Dose of Vaccination**

Category	n/N (%)	Crude analysis			Multivariate model 1 <sup>a</sup>			Multivariate model 2 <sup>b</sup>		
		OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P
Body mass index before pregnancy										
<19.2	41/46 (89)	1.00			1.00			1.00		
19.2–21.4	39/48 (81)	0.53	(.16–1.72)	0.29	0.80	(.21–3.07)	0.74	0.78	(.20–3.09)	0.72
$\geq$ 21.5	41/44 (93)	1.67	(.37–7.44)	0.50	2.36	(.46–12.3)	0.31	1.65	(.31–8.86)	0.56
		(Trend <i>P</i> = .58)			(Trend <i>P</i> = .29)			(Trend <i>P</i> = .58)		
Trimester										
First	18/22 (82)	1.00			1.00			1.00		
Second	37/43 (86)	1.37	(.34–5.47)	0.66	2.07	(.46–9.33)	0.34	2.21	(.48–10.2)	0.31
Third	66/73 (90)	2.10	(.55–7.96)	0.28	3.18	(.71–14.3)	0.13	3.35	(.72–15.6)	0.12
		(Trend <i>P</i> = .26)			(Trend <i>P</i> = .14)			(Trend <i>P</i> = .14)		
Age at H1N1 vaccination, years										
<29	38/43 (88)	1.00			1.00			1.00		
29–33	40/47 (85)	0.75	(.22–2.57)	0.65	0.80	(.20–3.18)	0.76	0.84	(.20–3.45)	0.81
$\geq$ 34	43/48 (90)	1.13	(.30–4.21)	0.85	1.04	(.24–4.55)	0.95	1.27	(.27–5.91)	0.76
		(Trend <i>P</i> = .84)			(Trend <i>P</i> = .94)			(Trend <i>P</i> = .74)		
2009–2010 seasonal influenza vaccination										
Unvaccinated	98/107 (92)	1.00			1.00					
Vaccinated	23/31 (74)	0.26	(.09–.76)	0.01	0.24	(.08–.76)	0.02			
Duration between seasonal vaccination and H1N1 vaccination										
Unvaccinated	98/107 (92)	1.00						1.00		
$\geq$ 20 days	14/16 (88)	0.64	(.13–3.29)	0.60				0.59	(.11–3.29)	0.55
$\leq$ 19 days	9/15 (60)	0.14	(.04–.48)	0.002				0.12	(.03–.48)	0.003
		(Trend <i>P</i> = .003)						(Trend <i>P</i> = .004)		
Prevaccination titer										
<1:10	78/92 (85)	1.00			1.00			1.00		
1:10–1:20	43/46 (93)	2.57	(.70–9.45)	0.16	2.82	(.69–11.5)	0.15	2.97	(.71–12.4)	0.14

**NOTE.** Logistic regression model. 138 study subjects were included for the analyses, because 11 subjects with prevaccination titer of  $\geq$ 1:40 were excluded. CI, confidence interval; OR, odds ratio.

<sup>a</sup> Model included body mass index before pregnancy, trimester, age at H1N1 vaccination, 2009–2010 seasonal influenza vaccination, and prevaccination titer.

<sup>b</sup> Model included body mass index before pregnancy, trimester, age at H1N1 vaccination, duration between seasonal and H1N1 vaccination, and prevaccination titer.

pregnancy, trimester, or age at vaccination and suggested that prior 2009–2010 seasonal influenza vaccination and a prevaccination titer to A/California (H1N1-pdm) might affect the immune responses to 2009 pandemic influenza A (H1N1) vaccination. The immunity after the first dose satisfied the international licensing criteria of the European Agency for the Evaluation of Medical Products and the US Food and Drug Administration. The second dose of vaccine conferred little additional induction of antibody. Previous studies showed that single dose of 2009 influenza A (H1N1) vaccine (with 15  $\mu$ g of antigen) achieved a protective level of antibody among 90%–97% of healthy adults, which is similar to the seroprotection level of 89% that was found in our study [13–16]. The results of the present study agree with a previous review that indicated that the antibody response to influenza vaccine was similar in pregnant and nonpregnant women [21].

An inverse association of the prevaccination titer with the fold rise and the seroresponse rate in serum HAI antibody, which is

known as the “law of initial values” or “negative feedback,” is clearly demonstrated in the present study [22]. These effects were independent of body mass index before pregnancy, trimester, age at vaccination, or the status of 2009–2010 seasonal influenza vaccination.

The 2009–2010 season, in which the present study was conducted, was an exceptional influenza season in that 2 types of influenza vaccine (2009 pandemic influenza A [H1N1] vaccine and 2009–2010 seasonal influenza vaccine) were prevailing because of the influenza A (H1N1) pandemic. In such a season, optimal timing of each vaccination might be a very important issue. Although the present study is limited because of a small sample size, the results might be useful in addressing this point. In the present study, the immune response to pandemic H1N1 vaccine was affected by recently received seasonal influenza vaccination, suggesting a potential interference in immune responses between the seasonal vaccination and pandemic influenza A/H1N1 vaccination. However, a previous study found

that the simultaneous administration of seasonal and pandemic H1N1 vaccine could induce sufficient levels of antibody to both the seasonal and the pandemic H1N1 vaccine strains [23]. Another study showed that when the seasonal and pandemic H1N1 vaccines were separately administered, the geometric mean titer level to the pandemic H1N1 vaccine strain was lower among the seasonal-vaccinated group than among the unvaccinated group, although the difference was not statistically significant [16]. Because there is still only limited evidence, further studies are necessary to examine the potential interference across influenza vaccines.

No severe adverse events occurred among pregnant women and their fetuses throughout the study period. One fetal death was reported on the day after vaccination; however, a pathologic diagnosis indicated that the fetal death had occurred  $\geq 7$  days before the H1N1 vaccination. Therefore, the fetal death was unrelated to the vaccination. Previous studies about the reactogenicity of seasonal influenza vaccine also reported no severe adverse events among fetuses and infants [24–26].

Because the present study was conducted during the peak of the pandemic wave in Japan, the following limitations should be discussed. The most important limitation might be the possibility of intercurrent asymptomatic infection. However, we monitored all subjects for influenza-like illness, and the 1 subject who experienced a confirmed influenza A virus infection (by the rapid test) between the first and second doses was excluded from the analyses. In addition, even when 6 subjects with influenza-like illness during the study periods were excluded from the analyses, the results were almost unchanged. Thus, we believe that the effect of intercurrent infection was not large enough to invalidate the present results.

At baseline, the proportion of subjects with a protective level of titers before vaccination was 7%, which is similar to that reported in China (4%) [13] but lower than that in Australia (27%) [14]. The proportion of subjects with protective levels of prevaccination titers could inevitably differ according to the location and time of the study, because these levels would be attributed to asymptomatic infections of the 2009 pandemic influenza A (H1N1) before recruitment or to cross-reactive antibodies induced by previous exposure (through infection or vaccination) to a virus that is genetically and antigenically similar to the 2009 pandemic influenza virus [27]. The stratified analyses by prevaccination titer performed in the present study are adequate to appropriately examine the immunogenicity of pandemic influenza A (H1N1) vaccine.

There have been few studies to examine the immunogenicity, safety, and effectiveness of the seasonal influenza vaccine among pregnant women. This lack of studies might contribute to the low vaccine coverage among pregnant women. However, the studies have shown that the influenza vaccination induces sufficient immune responses [28, 29], protects women from febrile respiratory illness [30–32], and does not cause severe adverse

events for pregnant women or their fetuses [24–26]. Additionally, studies have suggested that the vaccination of pregnant women could confer the beneficial effect for their infants by transfer of acquired antibodies through cord blood and could protect infants  $< 6$  months old from febrile respiratory illnesses, including influenza infection [28–32]. We anticipate that additional scientific evidence will help to appreciate the necessity of influenza vaccination and to increase vaccine coverage among pregnant women.

In conclusion, the present study indicated the immunogenicity of a single dose of H1N1 vaccination among pregnant women. No severe adverse events occurred among the participants. The potential interference between H1N1 vaccination and seasonal vaccination needs to be more thoroughly investigated in a different study setting to prepare for future influenza pandemics.

## Funding

This study was supported by Research on Emerging and Re-emerging Infectious Diseases, Health and Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare, Japan (grant H20-SHINKO-IPPAN-002).

## References

1. Harris JW. Influenza occurring in pregnant women: a statistical study of thirteen hundred and fifty cases. *JAMA* **1919**; 72:978–80.
2. Freeman DW, Barno A. Deaths from Asian influenza associated with pregnancy. *Am J Obstet Gynecol* **1959**; 78:1172–5.
3. Naleway AL, Smith WJ, Mullooly JP. Delivering influenza vaccine to pregnant women. *Epidemiol Rev* **2006**; 28:47–53.
4. Jamieson DJ, Honein MA, Rasmussen SA, et al. the Novel Influenza A (H1N1) Pregnancy Working Group. H1N1 2009 influenza virus infection during pregnancy in the USA. *Lancet* **2009**; 374:451–8.
5. 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.
6. Neuzil KM, Reed GW, Mitchel EF, Simonsen L, Griffin MR. Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. *Am J Epidemiol* **1998**; 148:1094–102.
7. Dodds L, McNeil SA, Fell DB, 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.
8. Cox S, Posner SF, McPheeters M, Jamieson DJ, Kourtis AP, Meikle S. Hospitalizations with respiratory illness among pregnant women during influenza season. *Obstet Gynecol* **2006**; 107:1315–22.
9. Lindsay L, Jackson LA, Savitz DA, et al. Community influenza activity and risk of acute influenza-like illness episodes among healthy unvaccinated pregnant and postpartum women. *Am J Epidemiol* **2005**; 163:838–48.
10. Hartert TV, Neuzil KM, Shintani AK, et al. Maternal morbidity and perinatal outcomes among pregnant women with respiratory hospitalizations during influenza season. *Am J Obstet Gynecol* **2003**; 189:1705–12.
11. Recommendations of the Advisory Committee on Immunization Practices (ACIP). Prevention and control of seasonal influenza with vaccines. *MMWR* **2009**; 58:12, 14–5, 24–33.
12. Broughton DE, Beigi RH, Switzer GE, Raker CA, Anderson BL. Obstetric health care workers' attitudes and beliefs regarding influenza vaccination in pregnancy. *Obstet Gynecol* **2009**; 114:981–7.

13. Liang XF, Wang HQ, Wang JZ, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet* **2010**; 375:56–66.
14. Greenberg ME, Lai MH, Hartel GF, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. *N Engl J Med* **2009**; 361:2405–13.
15. Zhu FC, Wang H, Fang HH, et al. A novel influenza A (H1N1) vaccine in various age groups. *New Engl J Med* **2009**; 361:2414–23.
16. Nolan T, McVernon J, Skeljo M, et al. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine in infants and children. *JAMA* **2010**; 303:37–46.
17. Hirota Y, Kaji M, Ide S, Goto S, Oka T. The hemagglutination inhibition antibody responses to an inactivated influenza vaccine among healthy adults: with special reference to the prevaccination antibody and its interaction with age. *Vaccine* **1996**; 14:1597–602.
18. Hirst GK. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. *J Exp Med* **1942**; 75:49–64.
19. European Committee for Proprietary Medical Products. Note for Guidance on Harmonisation of Requirements for Influenza Vaccines (CPMP/BWP/214/96). London: European Agency for the Evaluation of Medical Products, 1997.
20. Center for Biologics Evaluation and Research. Guidance for Industry: Clinical Data Needed To Support the Licensure of Pandemic Influenza Vaccines. Bethesda, MD: Food and Drug Administration, 2007.
21. 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.
22. Hobson D, Baker FA, Curry RL. Effects of influenza vaccines in stimulating antibody in volunteers with prior immunity. *Lancet* **1973**; 2:155–6.
23. Vajo Z, Tamas F, Sinka L, Jankovics I. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines when administered alone or simultaneously with the seasonal influenza vaccine for the 2009-10 influenza season: a multicentre, randomised controlled trial. *Lancet* **2010**; 375:49–55.
24. Munoz FM, Greisinger AJ, Wehmanen OA, et al. Safety of influenza vaccination during pregnancy. *Am J Obstet Gynecol* **2005**; 192:1098–106.
25. Deinard AS, Ogburn P. A/NJ/8/76 influenza vaccination program: effects on maternal health and pregnancy outcome. *Am J Obstet Gynecol* **1981**; 140:240–5.
26. 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**; 2:229–35.
27. Plennevaux E, Sheldon E, Blatter M, Reeves-Hoché MK, Denis M. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomized controlled phase 2 trials. *Lancet* **2010**; 375:41–8.
28. Englund JA, Mbawuike IN, Hammill H, Holleman MC, Baxter BD, Glezen WP. Maternal immunization with influenza or tetanus toxoid vaccine for passive antibody protection in young infants. *J Infect Dis* **1993**; 168:647–56.
29. Sumaya CV, Gibbs RS. Immunization of pregnant women with influenza A/New Jersey/76 virus vaccine: reactogenicity and immunogenicity in mother and infant. *J Infect Dis* **1979**; 140:141–6.
30. Zaman K, Roy E, Arifeen SE, et al. Effectiveness of maternal influenza immunization in mothers and infants. *New Engl J Med* **2008**; 359:1555–64.
31. Black SB, Shinefield HR, France EK, Fireman BH, Platt ST, Shay D; the Vaccine Safety Datalink Workgroup. Effectiveness of influenza vaccine during pregnancy in preventing hospitalizations and outpatient visit for respiratory illness in pregnant women and their infants. *Am J Perinatol* **2004**; 21:333–9.
32. Reuman PD, Ayoub EM, Small PA. Effect of passive maternal antibody on influenza illness in children: a prospective study of influenza A in mother-infant pairs. *Pediatr Infect Dis J* **1987**; 6:398–403.

