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Effects of prior influenza virus vaccination on maternal antibody responses: Implications for achieving protection in the newborns

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ABSTRACT

Background: In the US, influenza vaccination is recommended annually to everyone ≥6 months. Prior receipt of influenza vaccine can dampen antibody responses to subsequent vaccination. This may have implications for pregnant women and their newborns, groups at high risk for complications from influenza infection.
Objective: This study examined effects of prior vaccination on maternal and cord blood antibody levels in a cohort of pregnant women in the US.
Study design: Influenza antibody titers were measured in 141 pregnant women via the hemagglutination inhibition (HAI) assay prior to receipt of quadrivalent influenza vaccine, 30 days post-vaccination, and at delivery (maternal and cord blood). Logistic regression analyses adjusting for age, BMI, parity, gestational age at vaccination, and year of vaccination compared HAI titers, seroprotection, and seroconversion in women with versus without vaccination in the prior year.
Results: Compared to those without vaccination in the previous year (n = 50), women with prior vaccination (n = 91) exhibited higher baseline antibody titers and/or seroprotection rates against all four strains after controlling for covariates. Prior vaccination also predicted lower antibody responses and seroconversion rates at one month post-vaccination. However, at delivery, there were no significant differences in antibody titers or seroprotection rates in women or newborns, and no meaningful differences in the efficiency of antibody transfer, as indicated by the ratio of cord blood to maternal antibody titers at the time of delivery.
Conclusion: In this cohort of pregnant women, receipt of influenza vaccine the previous year predicted higher baseline antibody titers and decreased antibody responses at one month post-vaccination against all influenza strains. However, prior maternal vaccination did not significantly affect either maternal antibody levels at delivery or antibody levels transferred to the neonate. This study is registered with the NIH as a clinical trial (NCT02148874).

1. Introduction

Seasonal influenza virus vaccination is recommended by the Centers for Disease Control (CDC) and the American Congress of Obstetricians and Gynecologists (ACOG) to all women without contraindications who are pregnant or will be pregnant during influenza season [1,2]. This recommendation reflects recognition that pregnant women are at high risk for complications, hospitalization, and death due to influenza infection [3–7]. It is now established that influenza immunization during pregnancy reduces risk of influenza infection in pregnant women [8,9]. Studies show no adverse effects of vaccination in relation to outcomes including, but not limited to, risk of preterm labor, C-section, or fetal malformation [10–13].

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Via transplacental antibody transfer, maternal vaccination also confers protection against influenza virus to the neonate [8,9,14–20]. Infants from 0–6 months have among the highest rates of influenza-associated complications with >1000 hospitalizations per 100,000 infants in the US [21]. Influenza vaccine is not approved for infants <6 months. Thus, maternal vaccination in pregnancy is the only currently recommended effective strategy for protecting infants younger than 6 months. Prospective studies of laboratory-confirmed influenza show that maternal vaccination can reduce risk of influenza infection in infants by up to 63% and reduce influenza severity in infected infants [8,9,14–20].

Of clinical relevance, prior receipt of influenza vaccine can lower antibody responses to subsequent vaccination. In a study of 796 children and adolescents (6–17 years), receipt of seasonal trivalent inactivated influenza vaccine in the prior year predicted lower antibody responses against A/H1N1 and A/H3N2 strains [22]. Data from animal and human studies suggest that these blunting effects of prior vaccination are linked with higher basal antibody titers among previously vaccinated individuals, which may interfere with B-cell signaling [23,24]. However, lower antibody responses among those with prior vaccination have also been observed among those who did not exhibit elevated baseline titers [25,26].

Given the consistently observed effects of prior vaccination on subsequent antibody responses, concern has been raised that annual vaccination may interfere with development of protective immunity in the context of a lethal pandemic subtype [27]. In relation to this concern, a study of 150 pregnant women who received monovalent 2009 influenza A (H1N1) vaccine during the flu pandemic found that those who had already received trivalent seasonal vaccine in the same year exhibited less robust antibody responses to the monovalent H1N1 vaccine [28]. However, overall, data on effects of prior vaccination on maternal antibody responses are limited. Moreover, data on how prior maternal vaccination may affect antibody levels in the neonate is unknown.

Addressing gaps in the literature, the current study examined effects of prior vaccine receipt on antibody responses to seasonal influenza vaccine in a cohort of pregnant women in the US. This study included 141 women who were assessed prior to and following receipt of seasonal influenza vaccine during the 2013–2014 and 2014–2015 influenza seasons. We examined potential differences in maternal antibody status at baseline (i.e., prior to vaccination), antibody responses at ~30 days post-vaccination, antibody maintenance to the time of delivery, and cord blood antibody levels, as a function of maternal vaccination in the previous year.

2. Materials and methods

2.1. Study design

Pregnant women were recruited largely from faculty, staff, and students at the Ohio State University (OSU) and OSU Wexner Medical Center (OSUWMC) based on voluntary response to advertisements placed in online campus newsletters (n = 73, 51.8% of the final analytic sample). Women were also recruited from the OSUWMC Prenatal Clinic and surrounding community of Columbus, Ohio. All participants in the study were informed that they could discontinue participation at any time with no penalty and no change in their future relationship with The Ohio State University. Data collection occurred from October 2013 to September 2015, vaccinations occurred between late August and late April each vaccination year. Participants received an influenza vaccine at the first study visit, and provided blood samples at all three study visits (baseline, ~30 days post-vaccination, and delivery). Cord blood was also collected at delivery. This study is registered with the NIH as a clinical trial (NCT02148874).

2.2. Participants

Exclusion criteria included, chronic conditions (e.g., cancer, systemic lupus erythematosus) with implications for immune function. To ensure adequate time for follow-up assessment prior to delivery, women were excluded if they were beyond 30 weeks completed gestation; women were eligible to enroll at any other stage of gestation. Women were excluded if they reported weight and measured height consistent with a pre-pregnancy body mass index (BMI) > 50, or did not intend to deliver at OSUWMC. Women reporting acute illness, such as cold- or influenza-like symptoms, or antibiotic use within ten days of a study visit were rescheduled. The current analyses focused on the effects of prior receipt of influenza vaccination per self-report. A total of 145 women were recruited. Four women were excluded from analyses because they were uncertain about influenza vaccination status in the prior year, resulting in an analytic sample of 141. Cord blood samples were unavailable from 25 participants in this analytic sample. Written informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorizations were obtained from all participants and each received modest compensation. The study was approved by the OSU Biomedical Institutional Review Board.

2.3. Demographics

Age, race/ethnicity, marital status, education level, annual household income, employment status, and number of prior births (parity) were collected by self-report. Pre-pregnancy body mass index (BMI; kg/m^2) was calculated using self-reported pre-pregnancy weight and measured height at the first visit.

2.4. Influenza virus vaccine

Women received the 2013–2014 or 2014–2015 seasonal influenza vaccination, depending on the year recruited. Strains present in the seasonal influenza vaccine during the two study years, and one year preceding are presented in Table 1. Quadrivalent inactivated influenza vaccine (IIV4) which includes an A/H1N1, A/H3N2, and two B strains was introduced during the 2013–2014 season. The first 15 women vaccinated in this study year received

Table 1
Influenza virus vaccine strains by year.

Influenza season		
2012–2013	2013–2014	2014–2015
A/California/7/2009 (H1N1)-like virus	A/California/7/2009 (H1N1)-like virus	A/California/7/2009 (H1N1)-like virus
A/Victoria/361/2011 (H3N2)-like virus	A/Victoria/361/2011 (H3N2)-like virus	A/Texas/50/2012 (H3N2)-like virus
B/Wisconsin/1/2010-like virus	B/Massachusetts/2/2012-like virus	B/Massachusetts/2/2012-like virus
N/A	B/Brisbane/60/2008-like virus	B/Brisbane/60/2008-like virus

Women were vaccinated during the 2013–2014 and 2014–2015 flu seasons. The first 15 women vaccinated received trivalent vaccine which did not include B/Brisbane. Information on the 2012–2013 vaccine is provided for comparison to strains present in the subsequent year.

trivalent vaccine and were thus excluded from analyses related to the B/Brisbane strain.

2.5. Hemagglutination inhibition assay (HAI)

HAI assays were performed with specific viruses relating to vaccine year. A/California/04/2009 (CA/09, pdmH1N1), viral stocks were propagated in the allantoic cavity of 10-day-old specific pathogen-free embryonated chicken eggs at 37 °C. Allantoic fluid was harvested, cleared by centrifugation, and stored at −80 °C. A/Texas/50/2012 (H3N2) and A/Victoria/361/2011 (H3N2) were propagated in MDCK cells. HAI titer was blindly determined based on vaccine year in accordance with World Health Organization guidelines [29]. Briefly, women and infant serum were treated with receptor destroying enzyme (RDE; Denka Seiken, Tokyo, Japan), followed by inactivation. RDE-treated sera were then incubated in duplicate with virus for 15 min at room temperature followed by incubation at 4 °C with 0.5% Turkey red blood cells. HAI titer was determined by reciprocal dilution of the last well.

2.6. Seroconversion and seroprotection rates

Serum samples from baseline, ~30 days post-vaccination, and delivery were assayed using the hemagglutination inhibition (HAI) assay against vaccine strains from the corresponding year. HAI antibody titers reported as <1:10 were valued at 1:5 for statistical purposes. Consistent with prior studies [30], seroconversion was defined as a 4-fold increase in antibody titers or a titer of ≥40 if the starting value was <10 at the one month follow-up visit. Maternal seroprotection rates were determined at each study visit, as defined by an antibody titer ≥1:40 [31]. Seroprotection cut-offs of both 1:40 and 1:110 were examined in relation to cord blood titers, as prior data in children (6 months to 6 years of age) indicate that the more conservative 1:110 antibody level is necessary in children to achieve similar rates of protection as observed with a titer of 1:40 in adults [32].

2.7. Statistical analyses

Women were categorized into two groups: receipt of seasonal influenza virus vaccine in the prior year or not. To examine differences between these groups on demographic characteristics, *t*-tests and chi-square tests were conducted. Because HAI titers were not normally distributed even after log-transformation, non-transformed data were examined using proportional odds ordinal logistic regression to compare HAI titers for each influenza strain at each timepoint between women with and without prior vaccination. Logistic regression analyses were conducted to examine the effects of prior vaccination receipt on rates of seroconversion and seroprotection. All analyses adjusted for maternal age, pre-pregnancy BMI, parity, gestational age at the time of vaccination, and year of vaccination. Analyses were conducted in SPSS 24.0 and SAS 9.4.

3. Results

3.1. Sample characteristics

Study visits occurred at baseline (mean gestational age = 18.3 - weeks, SD ± 7.1 weeks), 30 days later (mean gestational age = 22.7 weeks, SD ± 7.2 weeks), and delivery (mean gestational age = 39.0 weeks, SD ± 1.7 weeks). Overall, 62.0% of women were White (n = 88), with a mean age of 29.2 years (SD ± 4.9). In this sample, 64.5% (n = 91) reported receiving an influenza vaccination in the previous year, while 35.5% (n = 50) did not. Women reporting vaccination in the previous year were older, with lower average BMI, higher education, higher income levels, and were more likely to be White, married, and employed (Table 2). Groups did not differ in parity or length of time from vaccination to delivery (Table 2).

Table 2
Demographic characteristics.

	No vaccine in prior year (n = 50)	Vaccine in prior year (n = 91)	p-value
Body Mass Index (BMI)			0.055
Normal (≥18.5–<25)	18 (36.0)	52 (57.1)	
Overweight (≥25–<30)	14 (28.0)	18 (19.8)	
Obese (≥30)	18 (36.0)	21 (23.1)	
Age [Mean (SD)]	27.7 (5.4)	30.0 (4.4)	0.007
Race [*n* (%)]			0.021
White	25 (50.0)	63 (69.2)	
Black	20 (40.0)	24 (26.4)	
Asian	1 (2.0)	2 (2.2)	
Multiracial	4 (8.0)	2 (2.2)	
Ethnicity [*n* (%)]			0.327
Hispanic	2 (4.1)	2 (2.2)	
Non-Hispanic	47 (95.9)	89 (96.7)	
Marital Status [*n* (%)]			0.001
Married	20 (40.8)	65 (71.4)	
In a relationship	19 (38.0)	18 (19.8)	
Single	11 (22.0)	8 (8.8)	
Education [*n* (%)]			<0.001
High School Graduate or Less	14 (28.0)	8 (8.8)	
Some College	19 (38.0)	19 (20.9)	
College Degree	9 (18.0)	28 (30.8)	
Graduate School	8 (16.0)	36 (39.6)	
Income [*n* (%)]			<0.001
<$29,999	34 (68.0)	20 (22.0)	
$30,000–74,999	9 (18.0)	28 (30.8)	
$ > 75,000	7 (14.0)	43 (47.3)	
Employment Status [*n* (%)]			<0.001
Employed outside the home	22 (44.0)	71 (78.0)	
Not employed outside the home	28 (56.0)	20 (22.0)	
Parity [*n* (%)]			0.860
0	22 (44.0)	28 (30.8)	
1	10 (20.0)	40 (44.0)	
≥2	18 (36.0)	23 (25.3)	
Trimester of Vaccination [*n* (%)]			0.296
First	18 (36.0)	28 (30.8)	
Second	29 (58.0)	52 (57.1)	
Third	3 (6.0)	11 (12.1)	
Weeks between Vaccination and Delivery [Mean (SD)]	21.0 (7.2)	20.6 (7.5)	0.73

3.2. Effects of prior vaccination on baseline antibody levels

Proportional odds ordinal logistic regression was used to compare HAI titers, adjusting for maternal age, pre-pregnancy BMI, parity, gestational age at time of vaccination, and year of vaccination. At baseline, women vaccinated the previous year had greater odds of having a higher HAI titer for A/H1N1 (p = 0.001), A/H3N2 (p = 0.04), and B/Massachusetts (p = 0.01), with a similar but non-significant trend for B/Brisbane (p = 0.12) compared to women not vaccinated the previous year (Fig. 1; Table 3). Per logistic regression utilizing the same covariates, women with prior vaccination had higher seroprotection rates (i.e., titer ≥1:40) at baseline for A/H1N1 (50.5% vs 32.0%, B = 1.00, SE = 0.42, p = 0.02), B/Massachusetts (40.7% vs 22.0%, B = 1.16, SE = 0.46, p = 0.01), and B/Brisbane strains (25.3% vs 6%, B = 2.67, SE = 1.10, p = 0.02). A similar, but non-significant trend was observed in relation to A/H3N2 (49.5% vs 38.0%, B = 0.63, SE = 0.40, p = 0.12).

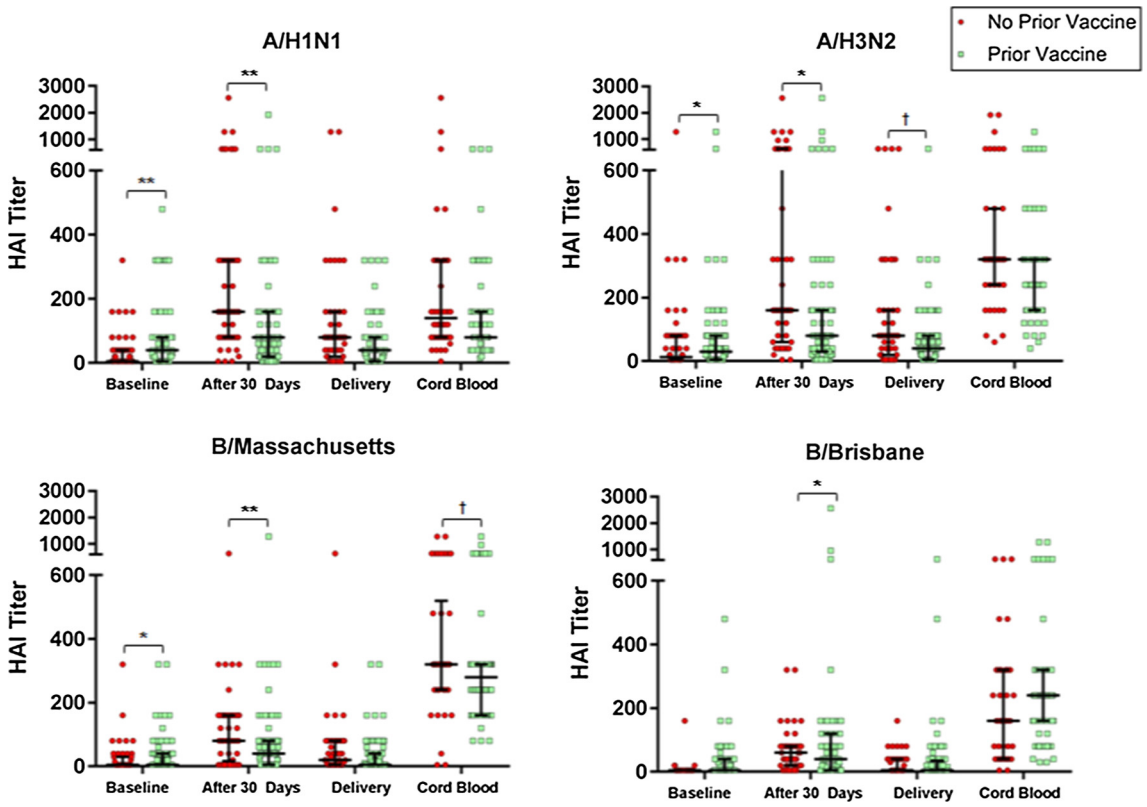

Fig. 1. Hemagglutination inhibition (HAI) titers for each influenza strain at each timepoint (Median; Interquartile Range). * $p < 0.05$, ** $p < 0.01$; † $p \leq 0.08$; analyses were conducted using proportional odds ordinal logistic regression controlling for maternal age, pre-pregnancy BMI, parity, gestational age at time of vaccination, and year of vaccination.

Table 3
Prior vaccination and proportional odds ratio for higher HAI titers.

Strain	Timepoint	Odds Ratio (95% CI)[a]	p-value
A/H1N1	Baseline	**3.46 (1.65, 7.27)**	**0.001**
	After 30 Days	**0.29 (0.14, 0.60)**	**0.001**
	Delivery	0.57 (0.28, 1.13)	0.11
	Cord Blood	0.67 (0.31, 1.45)	0.31
	Neonate:Maternal Ratio	2.11 (0.99, 4.52)	0.053
A/H3N2	Baseline	**2.11 (1.03, 4.30)**	**0.04**
	After 30 Days	**0.38 (0.19, 0.78)**	**0.01**
	Delivery	0.52 (0.26, 1.05)	0.07
	Cord Blood	0.55 (0.25, 1.20)	0.13
	Neonate:Maternal Ratio	1.49 (0.71, 3.17)	0.29
B/Massachusetts	Baseline	**2.97 (1.32, 6.70)**	**0.01**
	After 30 Days	**0.33 (0.16, 0.69)**	**0.003**
	Delivery	0.71 (0.34, 1.48)	0.36
	Cord Blood	0.50 (0.23, 1.09)	0.08
	Neonate:Maternal Ratio	1.00 (0.45, 2.22)	0.99
B/Brisbane	Baseline	2.16 (0.83, 5.65)	0.12
	After 30 Days	**0.43 (0.21, 0.89)**	**0.02**
	Delivery	1.05 (0.47, 2.35)	0.91
	Cord Blood	0.77 (0.36, 1.65)	0.50
	Neonate:Maternal Ratio	0.57 (0.25, 1.27)	0.17

Analyses control for maternal age, pre-pregnancy BMI, parity, gestational age at vaccination, and year of vaccination.
The bold numbers are all statistically significant at $p < 0.05$.
[a] Odds ratio >1 indicates that prior vaccination predicted greater odds of higher HAI titer/higher neonate:maternal ratio. Odds ratio <1 indicates that prior vaccination predicted lower odds of a higher HAI titer/higher neonate:maternal ratio.

3.3. Effects of prior vaccination on maternal antibody responses

Proportional odds ordinal logistic regression adjusting for specified covariates demonstrated that at one month post-vaccination, women with prior vaccination had lower odds of having a higher HAI titer for all four strains: A/H1N1 ($p = 0.001$), A/H3N2 ($p = 0.01$), B/Massachusetts ($p = 0.003$), B/Brisbane ($p = 0.02$) (Fig. 1, Table 3). Similarly, prior vaccination was associated with lower rates of seroconversion across all four strains: A/H1N1 (16.1% vs 78.3%, $B = -2.99$, $SE = 0.52$, $p < 0.001$), A/H3N2 (31.0% vs 71.7%, $B = -1.79$, $SE = 0.46$, $p < 0.001$), B/Massachusetts (14.9% vs 60.9%, $B = -2.17$, $SE = 0.47$, $p < 0.001$), and B/Brisbane (25.3% vs 65.2%, $B = -2.11$, $SE = 0.52$, $p < 0.001$) (Table 4). Finally, prior vaccination predicted lower seroprotection rates for A/H3N2 (74.7% vs 93.5%, $B = -1.46$, $SE = 0.67$, $p = 0.03$), B/Massachusetts (52.9% vs 73.9%, $B = -1.02$, $SE = 0.48$, $p = 0.03$), and B/Brisbane (52.0% vs 69.6%, $B = -1.09$, $SE = 0.50$, $p = 0.03$) at 30 days post-vaccination (Table 4). A similar trend was observed for A/H1N1 which was not statistically significant (71.3% vs 91.3%, $B = -1.13$, $SE = 0.61$, $p = 0.07$).

3.4. Effects of prior vaccination on maternal antibody maintenance at delivery

Proportional odds ordinal logistic regression adjusting for specified covariates demonstrated that at the time of delivery, prior vaccination did not affect HAI antibody titers against any strain (Fig. 1, Table 3), although a trend toward such an effect was observed for A/H3N2 ($p = 0.07$). Moreover, adjusting for specified covariates, seroprotection at delivery did not differ significantly among women with versus without prior vaccination for all four strains (Table 4): A/H1N1 (51.1% vs 72.3%, $B = -0.53$, $SE = 0.42$, $p = 0.21$), A/H3N2 (60.2% vs 72.3%, $B = -0.48$, $SE = 0.44$, $p = 0.27$), B/Massachusetts (39.8% vs 46.8%, $B = -0.11$, $SE = 0.43$, $p = 0.80$), or B/Brisbane (25.0% vs 29.8%, $B = -0.10$, $SE - 0.50$, $p = 0.85$).

Table 4
Maternal seroconversion and seroprotection in relation to prior vaccination.

	No vaccine in prior year (n = 50)	Vaccine in prior year (n = 91)	p-value
A/H1N1			
Seroconversion [*n* (%)]	36/46 (78.3)	14/87 (16.1)	<0.001
Seroprotection [n (%)]			
Baseline	16/50 (32.0)	46/91 (50.5)	0.02
After 30 days	42/46 (91.3)	62/87 (71.3)	0.07
At delivery	34/47 (72.3)	45/88 (51.1)	0.21
H3N2			
Seroconversion [*n* (%)]	33/46 (71.7)	27/87 (31.0)	<0.001
Seroprotection [n (%)]			
Baseline	19/50 (38.0)	45/91 (49.5)	0.12
After 30 days	43/46 (93.5)	65/87 (74.7)	0.03
At delivery	34/47 (72.3)	53/88 (60.2)	0.27
B/Massachusetts			
Seroconversion [*n* (%)]	28/46 (60.9)	13/87 (14.9)	<0.001
Seroprotection [n (%)]			
Baseline	11/50 (22.0)	37/91 (40.7)	0.01
After 30 days	34/46 (73.9)	46/87 (52.9)	0.03
At delivery	22/47 (46.8)	35/88 (39.8)	0.80
B/Brisbane			
Seroconversion [*n* (%)]	30/46 (65.2)	18/75 (25.3)	<0.001
Seroprotection [n (%)]			
Baseline	3/50 (6.0)	20/79 (25.3)	0.02
After 30 days	32/46 (69.6)	39/75 (52.0)	0.03
At delivery	14/47 (29.8)	19/76 (25.0)	0.85

Analyses control for maternal age, pre-pregnancy BMI, parity, gestational age at vaccination, and year of vaccination.

Table 5
Ratio of log-transformed HAI titers in infant cord blood to mother at delivery.

Strain	No vaccine in prior year	Vaccine in prior year
A/H1N1	1.17 (1.06, 1.58)[a]	1.37 (1.18, 2.07)[c]
A/H2N3	1.31 (1.14, 1.90)[a]	1.38 (1.23, 2.56)[c]
B/Massachusetts	1.66 (1.31, 2.84)[b]	1.82 (1.47, 2.84)[d]
B/Brisbane	2.26 (1.47, 3.06)[b]	2.56 (1.57, 2.84)[d]

Median and interquartile range.
[a] n = 38.
[b] n = 36.
[c] n = 76.
[d] n = 68.

3.5. Effects of prior vaccination on cord blood antibody titers

Proportional odds ordinal logistic regression adjusting for specified covariates demonstrated that prior vaccination did not affect cord blood antibody titers against any strain (Fig. 1; Table 3) although there was a trend for B/Massachussets, with lower titers among those with prior vaccination (p = 0.08). The ratio of infant:-maternal antibody titers at the time of delivery are shown in Table 5. Proportional odds logistic regression adjusting for specified covariates also showed no significant differences in the infant:maternal antibody ratio among women with versus without vaccination in the prior year (Table 3), although a trend was observed for a higher ratio among women with prior vaccination for A/H1N1 (p = 0.052).

Overall, 95–100% of infants achieved a cord blood titer of 1:40 against each strain (Table 6), thus no statistical analyses were conducted in relation to achieving this titer. Utilizing the more conservative clinical cut-off of 1:110 titer, no significant effects of prior vaccination were observed (Table 6): A/H1N1 (42.3% vs 65.8%, B = −0.75, SE = 0.47, p = 0.11), A/H3N2 (93.6% vs 92.1%, B = 0.72, SE = 0.91, p = 0.43), B/Massachusetts (96.2% vs 92.1%, B = 0.78, SE = −0.91, p = 0.39), and B/Brisbane strain (82.9% vs 57.9%, B = 0.21, SE = 0.79, p = 0.79).

Table 6
Prior Vaccination and Cord Blood Antibody Titers.

	No vaccine in prior year (n = 38)	Vaccine in prior year (n = 78)	p-value
A/H1N1			
1:40 [*n* (%)]	37/38 (97.4)	76/78 (97.4)	0.983
1:110 [*n* (%)]	25/38 (65.8)	33/78 (42.3)	0.11
A/H3N2			
1:40 [*n* (%)]	38/38 (100.0)	78/78 (100.0)	n/a
1:110 [*n* (%)]	35/38 (92.1)	73/78 (93.6)	0.43
B/Massachusetts			
1:40 [*n* (%)]	36/38 (94.7)	78/78 (100.0)	0.105
1:110 [*n* (%)]	35/38 (92.1)	75/78 (96.2)	0.39
B/Brisbane			
1:40 [*n* (%)]	35/38 (92.1)	68/70 (97.1)	0.234
1:110 [*n* (%)]	22/38 (57.9)	58/70 (82.9)	0.79

Analyses control for maternal age, pre-pregnancy BMI, parity, gestational age at vaccination, and year of vaccination; cord blood data were unavailable for 25 women; in addition, among those with cord blood samples, 8 did not receive the vaccine for B/Brisbane and were not included in related analyses.

3.6. Comment

These data show that pregnant women who received influenza vaccination in the previous year exhibited higher baseline antibody titers as well as diminished antibody responses to influenza vaccination in comparison to pregnant women without prior vaccination. This effect was observed across all four virus strains. These findings are consistent with studies in other populations that demonstrated higher baseline titers and lower antibody responses as a function of prior vaccination. For instance, among 61 healthy adults 22–49 years of age, those who received seasonal influenza virus vaccine in the prior year had higher baseline HAI titers as well as lower antibody responses [23]. Similarly, in a study of 158 elderly frail adults who were examined across sequential vaccination years, prior vaccination predicted both higher basal HAI titers in the subsequent year as well as slight impairment in post-vaccination responses [24]. Further, in a study of 57 hospital workers, prior vaccination predicted poorer antibody responses across strains, although elevations in baseline antibody titers were only observed for some strains [25]. Overall, existing data, including that from the present study, suggest that elevations in baseline antibody titers as a result of prior exposures to influenza antigens dampen the B-cell response to vaccination.

Interestingly, despite these baseline and one month post-vaccination differences between "naïve" subjects and women with prior vaccination history, HAI antibody titers or rates of seroprotection were not significantly different at the time of delivery. Furthermore, the percentage of infants achieving cord blood titer of either 1:40 or the more conservative clinical cut-off of 1:110 did not differ based on maternal vaccination the previous year. Moreover, no statistically significant differences were observed in the ratio of infant to maternal antibody levels at the time of delivery. Of note, the efficiency of antibody transfer was greater for B strains, as shown in Table 5. While previous studies have shown that antibody levels decline progressively within the first few months after vaccination [33], it is unclear why antibody levels appear to decline faster in the women receiving a primary compared to repeated vaccination course. It could be possible that the differences in rate of antibody decline may represent an immunological "baseline" for the pregnant host. Following the short-lived response, the total possible number of long-lived plasma B cells have reached survival niches in the bone marrow and may dictate antibody persistence

[34]. However, these questions are outside the scope of this work and will be the focus of future studies in pregnant women and other high-risk populations.

The current study provides novel evidence that, despite these observed differences in baseline and one month post-vaccination antibody titers, by the time of delivery, maternal antibody titers do not differ significantly and infants do not differ in attaining a protective antibody titer against any influenza strain. In these analyses, in addition to a titer of 1:40, a titer of 1:110 was examined as a more conservative clinical cut-off for antibody protection in neonates. Evidence suggests that in children a titer of 1:110 is required to achieve 50% clinical protection against infection, while the conventional adult cut-off of 1:40 is associated with only 22% protection [32]. Across strains, 95–100% of infants achieved a titer of 1:40 while 50–93% achieved a titer of 1:110. These comparatively high titer values in cord blood versus maternal serum reflect active transport mechanisms; cord blood antibodies including influenza-specific IgG typically considerably exceed maternal levels [35–37], as observed in the current study. However, there are a number of factors that can impede transfer of antibody, and thus higher titers in neonates versus mothers is not observed in all studies [9].

Although infants can also acquire anti-influenza antibody via breast milk [38], breastfeeding rates in the US are low. It is estimated that only 41.7% of women breastfeed for 6 months or greater, including those who supplement with formula, with significantly lower rates among Black (26.6%) versus non-Hispanic White women (43.2%) [39]. For this reason, adequate transplacental antibody transfer is of primary clinical value. Thus, it is of clinical importance that the current data indicate that prior maternal vaccination does not impair antibody protection conferred to the neonate by maternal vaccination during the current pregnancy.

One interesting observation from this study is that overall level of influenza antibody appears to return to the maternal "baseline" between vaccination and delivery. Since placental antibody transfer is maximal in the third trimester, the decline to maternal baseline could suggest that the infant may receive less influenza antibodies due to vaccinating the mother in the first trimester. However, placental transfer occurs as an active transport process throughout pregnancy and transfer of antibodies from maternal to fetal blood increases with the duration after vaccination [40–43]. Several studies have also shown that vaccination late in pregnancy (15–28 days before delivery) does not increase antibody titers in the newborn [42,43]. In addition to maximal antibody transfer, vaccinating against influenza early in pregnancy is equally important for protection of both the mother and the fetus against currently circulating seasonal strains. Indeed, influenza vaccination during the first trimester has been shown to reduce the rate of stillbirth, premature delivery, and neonatal death [44,45]. Therefore, maternal influenza vaccination within the first trimester is vitally important not only for maximal antibody transfer but also for protection of the newborn and mother. If the mother were not vaccinated during pregnancy, not only would this increase chance of influenza infection and complications with birth but it may also result in decreased antibody transfer.

Demographic differences were observed between groups as would be expected. Specifically, women with prior vaccination had higher household incomes and lower BMI. These findings correspond with epidemiological data on vaccine uptake showing that adults of higher socioeconomic status (SES) with better health behaviors are more likely to receive seasonal influenza vaccines [46]. With regard to age, women with prior vaccination were older, reflecting the association between higher SES with later childbearing [47]. However, all women in the study were between 19 and 42 years old. With a mean age of 30.0 versus 27.7 years among those with versus without prior vaccination, the age difference between groups was minimal. Although aging is associated with impaired antibody responses to influenza vaccines, such effects are observable in the context of studies in elderly adults [48–50]. Moreover, all analyses accounted for effects of key covariates including maternal age, pre-pregnancy BMI, parity, gestational age at the time of vaccination, and year of vaccination.

Our study has limitations. Vaccination history was based on self-report rather than medical record. This introduces possible error. However, reporting of prior vaccination among medical center employees, who made up a large portion of this sample, is likely accurate given that vaccination is a requirement of employment. This study did not capture whether women with prior vaccination received inactivated or live-attenuated form at the time of prior vaccination. In this study, we determined vaccination in the prior year only. Repeated annual vaccination is common in certain groups, including the medical center faculty and staff who comprised a large part of our recruitment pool. It is unknown if the effects observed in the current study are due predominately to vaccine received in the immediate year preceding, or if this effect may be magnified by receipt over multiple years. As such, this study suggests that, even in the context of repeated prior vaccination, antibody protection conferred to the neonate is likely unaffected.

This study was conducted in central Ohio in a predominately White and African American sample. It is possible effects would differ in other contexts or populations. Relatedly, this study was conducted in generally healthy women. Prior data demonstrate that among women with human immunodeficiency virus (HIV) infection, lower maternal antibody titers are observed at one month post-vaccination as well as lower cord blood antibody titers in infants at delivery [43]. The effects of prior vaccination within women with chronic health conditions may differ from results observed in the current healthy cohort.

Available data clearly demonstrate that the clinical protection provided by influenza vaccines is closely associated with their immunogenicity [51]. Thus, for influenza vaccines, vaccine-induced HAI antibody titers are widely recognized a good surrogate indicator of clinical efficacy in healthy adults [51]. However, rates of clinically confirmed influenza infection were not examined in this study. As reviewed, attenuated immunological responses have been demonstrated in several studies in relation to repeated vaccination [23,52,53]. Corresponding impairment in vaccine efficacy (as defined by rates of clinical infection), while observed in some studies [54], have not consistently been reported [55,56]. Thus, the ultimate clinical implications of the attenuated immune responses among women with prior vaccination for disease susceptibility and severity are unknown. Given their high risk status, this question should be addressed empirically in a larger prospective cohort of pregnant women.

In sum, this investigation confirms prior findings showing that previous receipt of influenza virus vaccine is associated with higher baseline antibody titers and less robust antibody responses to subsequent vaccination in various populations. Our data also extend prior literature by showing that, differences at baseline and one month post-vaccination, prior vaccination does not significantly affect maternal antibody levels by the time of delivery *or* antibody levels in neonate (per cord blood). Thus, the current study indicates that prior vaccination does not significantly affect the antibody protection conferred to neonates by maternal vaccination in the current pregnancy.

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Conflicts of interest

The authors report no potential conflicts of interest. Octavio Ramilo, reports personal fees from HuMabs, Abbvie, Janssen, Medimmune and Regeneron, and grants from Janssen. All these fees and grants are not related to the current work.

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