Assessing the reactogenicity of Tdap vaccine administered during pregnancy and antibodies to Bordetella pertussis antigens in maternal and cord sera of Thai women

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Abstract

Introduction

Pregnant Thai women have low antibody titers against *B. pertussis* antigens, which coincide with an increasing incidence of pertussis among Thai infants. Thus, there exists a potential benefit of a booster dose of tetanus- diphtheria-acellular pertussis (Tdap) vaccine administered during pregnancy. Here, we report the vaccine reactogenicity profile and birth outcomes in Tdap-vaccinated pregnant women who have or have not had prior immunization with tetanus vaccine, and the IgG levels to *B. pertussis* antigens in maternal and cord sera at delivery.

Materials and methods

Pregnant women (N=370) aged 18-40 years were administered the Tdap vaccine (Boostrix®, GlaxoSmithKline, Rixensart, Belgium) at 26 to 36 weeks gestation. Adverse events following vaccination were identified by follow-up telephone call and medical record review. IgG against pertussis toxin (anti-PT), filamentous hemagglutinin (anti-FHA) and pertactin (anti-PRN) in both maternal and umbilical cord blood obtained at delivery were quantitatively evaluated using enzyme-linked immunosorbent assay (EUROIMMUN®, Lübeck, Germany).

Results

There was no reported increase in the severity or duration of adverse events associated with the administration of an extra tetanus vaccine within the previous five years (N=181) or multiple doses of tetanus vaccine during the current pregnancy (N=98). Vaccination at least eight weeks prior to delivery resulted in high antibody titers to all *B. pertussis* antigens studied.

Conclusions

The reactogenicity of Tdap vaccine administered during pregnancy was not affected by prior tetanus toxoid immunization. High transplacental antibody against *B. pertussis* antigens in the

cord blood provides evidence of antibody transfer and should thus help to protect newborns from pertussis during early life.

*Title Page

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Nasamon Wanlapakorn[1,2], Kirsten Maertens[3], Surasith Chaithongwongwatthana[4], Donchida Srimuan[1], Narissara Suratannon[1], Sompong Vongpunsawad[1], Thao Mai Phuong Tran[5], Niel Hens[5,6], Pierre Van Damme[3], Camille Locht[7], Yong Pooworavan[1*] & Elke Leuridan[3*]

[1]Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[2]Joint PhD program in Biomedical Sciences and Biotechnology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[3]Center for the Evaluation of Vaccination, Vaccine & Infectious Disease Institute, Faculty of Medicine and Health Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[4]Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[5]Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Belgium

[6]Centre for Health Economics Research & Modelling Infectious Diseases, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium

[7]Univ. Lille, CNRS, INSERM, Institut Pasteur de Lille, U1019 - UMR8204, Center for Infection and Immunity of Lille

*These two authors share the last authorship.

Corresponding author: Professor Yong Poovorawan

Email: yong.p@chula.ac.th

Postal address: Center of Excellence in Clinical Virology

Department of Pediatrics, Faculty of Medicine, Chulalongkorn University

1873 Rama IV road, Pathumwan, Bangkok 10330, Thailand

Tel: 66 2 256-4909; Fax: 66 2 256-4929

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Nasamon Wanlapakorn[1,2], Kirsten Maertens[3], Surasith Chaithongwongwatthana[4], Donchida Srimuan[1], Narissara Suratannon[1], Sompong Vongpunsawad[1], Thao Mai Phuong Tran[5], Niel Hens[5,6], Pierre Van Damme[3], Camille Locht[7], Yong Pooworavan[1*]& Elke Leuridan[3*]

[1]Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[2]Joint PhD program in Biomedical Sciences and Biotechnology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[3]Center for the Evaluation of Vaccination, Vaccine & Infectious Disease Institute, Faculty of Medicine and Health Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[4]Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[5]Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Belgium

[6]Centre for Health Economics Research & Modelling Infectious Diseases, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium

[7]Univ. Lille, CNRS, INSERM, Institut Pasteur de Lille, U1019 - UMR8204, Center for Infection and Immunity of Lille

*These two authors share the last authorship.

Corresponding author: Professor Yong Poovorawan

Email: yong.p@chula.ac.th

Postal address: Center of Excellence in Clinical Virology

25 Department of Pediatrics, Faculty of Medicine, Chulalongkorn University

26 1873 Rama IV road, Pathumwan, Bangkok 10330, Thailand

27 Tel: 66 2 256-4909; Fax: 66 2 256-4929

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Keywords: Pertussis, Vaccine, Pregnancy, Reactogenicity, Safety, Thailand

1. Introduction

Pertussis is a respiratory disease caused by the gram-negative bacteria *Bordetella pertussis*. Although pertussis is vaccine preventable, new infection readily occurs in both developed and developing countries despite the implementation of vaccination efforts worldwide. Severe morbidity and mortality associated with pertussis often occurs in infants and young children [1]. Current pertussis immunization strategies fail to protect infants who are too young to have received their primary series of pertussis vaccination. These infants are susceptible to severe pertussis-related complications and even death due to the lack of protective immunity.

Passively acquired maternal *B. pertussis*-specific antibodies are relatively low and transient in newborns despite an active transplacental transport [2]. Infants born to Tdap-vaccinated mothers had significantly higher titers of *B. pertussis* antibodies than those born to unvaccinated mothers [3]. Consequently, many countries including the UK, USA, Spain, Italy, Belgium and Argentina have implemented the Tdap vaccination during pregnancy in their national immunization programs in order to increase the maternal *B. pertussis* antibody levels which will be transplacentally transferred to protect the newborn during the first months of life [4-6]. High titers of naturally-acquired maternal-derived *B. pertussis* antibodies have been shown to interfere with the infant humoral immune response induced by the whole cell pertussis (wP) but not to acellular pertussis (aP) vaccine [7]. In contrast, an interference has been observed in maternal-derived Tdap-induced anti-*B. pertussis* antibody in aP-vaccinated infants in clinical studies from the US, Belgium and Vietnam [8-12].

At the April 2014 World Health Organization meeting by the Strategic Advisory Group of Experts on immunization to prevent early mortality, researchers concluded that data required

for the implementation of maternal Tdap immunization in countries where wP vaccine is used in infant vaccination programs could not be derived from the extrapolated aP vaccine data. Tdap-induced maternal antibodies may interfere with infant immune response induced by wP vaccine. Moreover, additional information on the safety and reactogenicity of repeated tetanus vaccination are vital to the effective implementation of pertussis immunization in countries with an existing tetanus vaccination during pregnancy such as Thailand.

The increasing incidence of pertussis in Thai infants [13], reportedly low antibody titers to *B. pertussis* antigens among Thai pregnant women [14] and the lack of data on potential blunting after wP vaccine administration in the presence of maternal antibodies, warrant the need to assess the effect of a booster dose of Tdap vaccination during pregnancy. Here, we report the reactogenicity profile of Tdap vaccine in a randomized controlled clinical trial involving Tdap-vaccinated Thai mothers, and describe the concentrations of *B. pertussis*-specific antibodies in paired maternal and umbilical cord sera. We also report on the adverse events and pregnancy outcomes when multiple tetanus vaccines are administered. Further studies regarding the interference of maternal-derived antibodies in wP-vaccinated infants are ongoing for this cohort.

2. Materials and methods

2.1 Study design

This study was conducted according to the Declaration of Helsinki and Good Clinical Practice Guidelines (ICH-GCP). It was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB no. 604/57) and the ethical committee of the University of Antwerp, Belgium. Written informed consent was obtained from all pregnant women prior to enrollment.

This prospective randomized controlled study involved pregnant women of Thai citizenship who were offered vaccination with Tdap vaccine administered between 26 and 36 weeks of gestation according to the US Advisory Committee on Immunization Practices (ACIP) recommendation [15]. Healthy pregnant women aged 18-45 years with low obstetrical risks (inclusion and exclusion criteria in Appendix 1) were recruited during routine antenatal visits at King Chulalongkorn Memorial Hospital in Bangkok between April 2015 and September 2016.

All healthy infants born after 36 weeks of gestation and weighed greater than 2,500 grams, were included for the follow-up study (ClinicalTrial.gov NCT02408926). Ten milliliters of blood were collected from pregnant women and the umbilical cord at delivery. Serum was separated from whole blood and stored at -20 °C prior to testing.

2.2 Study vaccine

Each 0.5 mL dose of the Tdap vaccine (Boostrix®, GlaxoSmithKline Biologicals, Rixensart, Belgium) contained 2.5 Lf of diphtheria toxoid (DT), 5 Lf of tetanus toxoid (TT), 8 µg of inactivated pertussis toxin (PT), 8 µg of formaldehyde-treated filamentous hemagglutinin (FHA) and 2.5 µg of formaldehyde-treated pertactin (PRN) adjuvanted with aluminium hydroxide. The vaccine was administered to pregnant women in the musculus deltoideus by the nurse or doctor.

2.3 Safety and reactogenicity

Acute adverse reaction was assessed in all women 30 minutes post-injection. Research nurses made follow-up telephone calls on day 2 and again on day 7 post-vaccination to record adverse events (AE) such as redness, pain, and induration at the site of injection, or fever.

Participants were encouraged to report possible AE anytime thereafter. In instances where AE were reported, daily follow-up telephone calls were made to record the severity and duration of AE until symptom resolution. Serious adverse events (SAE) and pregnancy outcome were recorded for all participants. AEs and SAEs that occurred after vaccination were evaluated jointly by the investigators and the data safety monitoring board.

2.4 Laboratory testing

The anti-PT, anti-FHA and anti-PRN IgG titers were analyzed using commercial ELISA kits (EUROIMMUN, Lübeck, Germany) according to the manufacturer's instructions. The ELISA kits used were calibrated based on the World Health Organization international standards. The values were expressed in International Units (IU) per milliliter. Serum samples were initially diluted 1:101 and further dilutions were made as needed to yield results within the detection range. Values below the lower limit of detection (< 5 IU/ml) observed in some samples were calculated as 50% of the cut-off values (2.5 IU/ml).

2.5 Statistical analysis

The number of pregnant women in this study was calculated based on the estimation of possible interference of maternal anti-PT in wP-vaccinated children [7] (significance level=0.05, power =0.90). The IgG levels were expressed as geometric mean concentrations (GMC) with 95% confidence interval. Data were analyzed using SPSS software version 24 (IBM Inc., Armonk, NY, USA) and R statistical software version 3.4.1. Pearson's correlation was used to show the relationship between maternal and cord antibody titers. The conventional t-test was performed on the antibody logarithmic scales to compare the GMC in pregnant women who received Tdap before and after 30 weeks gestation [16]. The t-test was also used to test the

difference in cord/maternal ratios of antibody levels in these two groups. Since the sample sizes of the two groups were reasonable high, we could rely on the central limit theorem and hence a conventional t-test would be valid [17]. To test the difference in the duration of solicited AE in women with or without prior tetanus immunizations, Mann-Whitney U test was employed. Assessment of factors affecting cord antibody levels was performed using a regression approach with the log-transformed values as the outcome. There were three steps: (1) variable selection using random forest; (2) backward model selection based on Akaike Information Criteria (AIC) using multiple linear regression and (3) further model reduction using likelihood ratio tests. This procedure was used before for model building [18]. Antibody titers below the detection limit (censoring observations) and two extreme outliers of anti-PRN cord-to maternal ratios were excluded.

3. Results

3.1 Demographic characteristics of the pregnant women and infants

A total of 631 pregnant women were screened, of whom 370 enrolled and were vaccinated with Tdap (Table 1). The majority of women (93%) delivered at full-term. There were 297 (80.3%) maternal blood and 284 (76.7%) cord blood samples collected at delivery.

3.2 Reactogenicity profile and pregnancy outcome after Tdap

No women reported AE within 30-minute post-vaccination. In the subsequent days following vaccination, pain was the most common AE reported (76.2%), most of which were mild (mean duration = 2.5 days) (Tables 2 and S1-S4). Low grade fever was the second most common AE (5.1%) (mean duration = 2.6 days). Swelling and redness were infrequent and generally resolved within a few days. SAE were 37 obstetrical, 4 fetal and 47 neonatal. Of 6.7 %

preterm deliveries, 84 % were late preterm (GA 34-37 weeks). There were 2 exclusions due to fetal deaths. None of the AE were determined to be related to vaccination.

3.3 Prior tetanus vaccination and risk of AE

Prior to enrolling in this study, 181 women recalled previously having received at least one dose of a tetanus vaccine within the past five years (1 dose in 51 women, 2 doses in 94 women, and 3 doses in 36 women). Ninety-eight women received at least one extra dose of tetanus vaccine during this current pregnancy (1 dose in 37 women, 2 doses in 60 women, 3 doses in 1 woman). Administration of prior tetanus vaccine within the previous 5 years (N=181 women) or during the current pregnancy (N= 98 women) did not increase the incidence and severity of any solicited AEs nor resulted in prolonged duration of the symptoms. There was also no observed increase in the occurrence of other AE or premature delivery among the women in this study (Table S5).

3.4 Differences in antibody titers at delivery

The geometric mean concentration (GMC) of anti-PT, anti-FHA and anti-PRN IgG were similar between maternal and cord blood pairs (Table 3). Maternal anti-PT, anti-FHA and anti-PRN IgG significantly correlated with cord values (Figure 1). In addition, when pregnant women were stratified into two groups based on their gestational age at vaccination, maternal anti-FHA IgG in the early Tdap group who had received Tdap between 26-30 weeks (n=194) was significantly lower than those vaccinated between 31-36 weeks (n=175) (p=0.024, t-test) (Figure 2). However, the cord-to-maternal antibody ratios were significantly higher for all three

antibodies in the early Tdap group than in the late Tdap group. Thus, differences in the cord-to-maternal ratios were seen depending on the time of vaccination and antigens analyzed.

3.5 Factors affecting anti-PT, anti-PRN and anti-FHA in cord sera.

Our analysis showed that maternal antibody titers at delivery and the interval between Tdap vaccination and delivery significantly affected the cord titers. This result is in agreement with the significant correlation between maternal antibody levels and cord values as shown in section 3.4 (Figure 1). Longer interval between vaccination and delivery led to higher titers of antibodies to *B. pertussis* antigens tested in the cord blood (Figure 3), particularly at 2-8 weeks prior to delivery. This effect decreased when vaccination was given between 8-14 weeks prior to delivery as demonstrated by the decrease in the steepness of the curve. Taken together, these findings suggest that vaccination at least eight weeks prior to delivery maximized antibody titers to all three *B. pertussis* antigens in the cord blood.

4. Discussion

Here, we report the first of many results from a prospective, randomized, controlled clinical trial examining the effect of Tdap vaccination during pregnancy on infant immune responses to aP and wP vaccines. Analysis of the antibodies to *B. pertussis* antigens as detected in the maternal and cord blood suggests that Tdap vaccination early in the third trimester (26-30 weeks) induced significantly higher cord-to-maternal ratios than vaccination later in the pregnancy (31-36 weeks). This is in agreement with other studies, which demonstrated the benefits of early vaccination. A study from Switzerland reported that anti-PT and anti-FHA antibodies in the cord sera were higher after the second trimester vaccination (up to 26 weeks of

gestation) than in the third trimester vaccination [19]. Abu Raya et al. also observed that vaccination at 27-30 weeks elicited a significantly higher anti-PT and anti-FHA antibody titers in cord blood than at 31 to 36 weeks [20]. Our study did not reveal any significant differences in the cord blood antibody titers among the early and the late Tdap groups, but we note the monotonic non-linear relationship between interval of vaccination-delivery and cord titers, which suggests that optimal antibody transfer occurs when women are vaccinated at least eight weeks prior to delivery.

We report a lower gradient of transplacentally transferred ratios of all *anti-B. pertussis* antibodies compared to prior studies in the USA [8] and Belgium [9], but comparable anti-FHA antibody cord-to-maternal ratios were reported from the study in Vietnam and Nepal [12, 21]. In the Belgian and Vietnamese studies, higher avidity of anti-PT antibodies in maternal and cord sera in Belgian mother-infant pairs was seen compared to Vietnamese mother-infant pairs. Thus, the efficiency of placental transfer may increase as a result of higher antibody avidity [22]. Avidity of the antibodies can depend on factors including the number of previous vaccine doses, the types of vaccine and natural exposure of the disease, all of which may influence the transport rate through the placenta and result in different cord-to-maternal antibody ratios among different cohorts. In addition, this study demonstrates that timing of vaccination also impact the cord-to-maternal antibody ratios, with significantly higher values similar to the US study in early third trimester vaccination [8]. Other factors such as genetics, maternal age and existing comorbidities could potentially affect the placental function and transplancental antibody transport, although there were no major morbidities in this Thai pregnant women cohort.

Variations in the transport ratios for antibodies to the different antigens were found in this study. The anti-PRN IgG cord-to-maternal ratio was lowest among the three anti-*B.pertussis*

antibodies studied here. This finding is consistent with previous observations [8-9]. This might be due to the characteristics of anti-PRN in terms of the variable proportions of IgG subclasses in response to the antigen and the functionality of the antibody [23, 24]. Future studies evaluating IgG subclasses and the antibody functionality may reveal the reasons for differences in transplacentally transferred ratios.

The presence of pre-existing antibodies from previous tetanus vaccine did not appear to exacerbate local or systemic adverse reactions nor affect the pregnancy outcomes among Tdap-vaccinees in our cohort. This is in agreement with other studies which found that Tdap vaccination during pregnancy was not associated with an increased risk of acute local and systemic reactions or adversely affected pregnancy outcomes in women receiving multiple doses of tetanus vaccines in a short period of time [25-26]. It also did not increase the risk of obstetrical and neonatal complications [27-32], although two studies suggested a slightly increased risk of chorioamnionitis in the Tdap-vaccinated group [33-34]. A US study observed that non-vaccinated women had higher preterm birth rates, incidence of small for gestational age, and length of neonatal hospitalization than Tdap-vaccinated mothers [25]. Our study observed that rates of obstetrical, neonatal and fetal SAE in Tdap-vaccinated pregnant women were similar or even lower compared to the general rate of AE and SAE reported at King Chulalongkorn Memorial Hospital, possibly due to the cohort bias. Our inclusion criteria strictly recruited women with low risk of obstetrical complications, which could explain the lower AE in Tdap-vaccinated group. As a tertiary care hospital and a referral center for complicated pregnancy, the incidence of obstetrical complications is likely to be higher compared to the general Thai population.

In conclusion, we demonstrated that Tdap vaccination during pregnancy was safe and well-tolerated even after recent tetanus vaccination. Our results also highlight that early vaccination was associated with high transplacental antibody transfer, which may help to protect newborn infants from pertussis during the first few months of life. Further studies examining the interference of maternal-derived antibodies in wP-vaccinated infants are ongoing.

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Conflict of Interest

All authors declared no conflict of interest.

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371 **Table 1.** Descriptive characteristics of participants in this study.

Characteristics	**Pregnant women (n=370)**
Mean Age (SD)	28.9 years (5.5)
Mean Gestational age at vaccination (SD)	30.7 weeks (2.3)
Mode of delivery	
Vaginal	209 (56.5%)
Cesarean	158 (42.7%)
No information	3 (0.8%)
Average days between vaccination and delivery (SD)	54.1 days (18.8)
Gestational age at delivery	
< 37 weeks	25 (6.7%)
≥ 37 weeks	344 (93.0%)
No information	1 (0.3)
Mean infant birth weight (SD)	3087.6 grams (416.2)

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Table 2. Summary of the adverse events (AE) and the severe adverse events (SAE) reported among Tdap-vaccinated pregnant women and neonates in this study.

	Type of reaction	Description	No. (%)	Reported among 4,636 deliveries at KCMH* in 2015 (%)
AE	Localized to the injection site	- Pain total	282 (76.2)	
		Mild	231	
		Moderate	51	
		Severe	0	
		- Swelling total	15 (4.1)	
		Mild	15	
		Moderate	0	
		Severe	0	
		- Redness total	5 (1.4)	

		Mild	5	
		Moderate	0	
		Severe	0	
	Systemic	- Low grade fever	19 (5.1)	
		- Upper respiratory tract infection	1 (0.3)	
		- Uterine contraction	1 (0.3)	
		- Rash	1 (0.3)	
		- Itchiness	1 (0.3)	
		- Vertigo	1 (0.3)	
		- Vomiting	1 (0.3)	
		- Chest discomfort	1 (0.3)	

	Others	-Gestational diabetes mellitus	10 (2.7)	8.8
		-Gestational hypertension	5 (1.4)	1.9
		-Thrombocytopenia	1 (0.3)	0.1
		-Oligohydramnios	3 (0.8)	0.8
SAE	Obstetrical	- Premature delivery	25 (6.7)	12.8
		- Premature contractions resulting in hospitalization	3 (0.8)	N/D
		- Chorioamnionitis	2 (0.5)	0.4
		- Psychosis at delivery	1 (0.3)	N/D
		- Severe pre-eclampsia	4 (1.1)	2.1
		- HELLP[a] syndrome	1 (0.3)	2.1
		- Urinary tract infection	1 (0.3)	2.0
	Fetal	- Fetal death	2 (0.5)	0.8

		- Congenital defects[b]	2 (0.5)	2.1[c]
	Neonatal	Neonatal birth asphyxia, No. (%)		
		- Severe birth asphyxia (APGAR score at 1 minute = 0-3)	2 (0.5)	1.3
		- Mild to moderate birth asphyxia (APGAR score at 1 minute = 4-7)	10 (2.7)	6.1
		- Prolonged hospitalization after birth[d]	35 (9.5)	21.0

*KCMH= King Chulalongkorn Memorial Hospital, AE = adverse events, SAE = severe adverse events, and N/D = no data.

[a]Hemolysis, elevated liver enzyme levels, and low platelet levels.

[b]One cleft lip and cleft palate and one imperforated anus.

[c]Conditions of significant morbidity, which required medical and/or surgical treatment.

[d]Defined as hospitalization >5 days after birth as a result of an illness.

Table 3. Geometric mean concentration of anti-PT, anti-FHA and anti-PRN IgG in maternal and cord blood from Tdap-vaccinated women.

IgG	Maternal, IU/ml (95%CI)	Cord, IU/ml (95%CI)	Ratio cord/maternal (95%CI)
Anti-PT	42.9 (38.5-47.7)	48.6 (43.5-54.4)	1.18 (1.13-1.23)
Anti-FHA	347.4 (304.5-396.3)	383.0 (336.9-435.4)	1.18 (1.12-1.24)
Anti-PRN	125.3 (99.1-158.4)	128.8 (101.8-162.9)	1.08 (1.03-1.13)*
Total	297	284	278

* After exclusion of two extreme outliers

Figure legends

Figure 1. Correlations of anti-PT (A), anti-FHA (B) and anti-PRN (C) IgG in maternal and cord sera. Pearson's correlation coefficient (r) for anti-PT = 0.89; p value <0.001, for anti-FHA=0.85; p value <0.001 and for anti-PRN = 0.86; p-value <0.001.

Figure 2. Antibody levels and cord-to-maternal ratios between early Tdap-vaccinated and late Tdap-vaccinated pregnant women. (a) Comparison of antibodies to PT, FHA and PRN. Maternal anti-FHA was significantly higher in the late Tdap group (*p=0.024, t-test). Maternal and cord sera in the early Tdap group, n = 152; maternal sera in the late Tdap group, n=145; cord sera in the late Tdap group, n =132. (b) cord-to-maternal ratios were

significantly higher in all three *B. pertussis*-specific antibodies among early the Tdap group (**p<0.001, t-test). Sample size in the early Tdap group, n = 147; sample size in the late Tdap group, n = 131. Error bars represented upper 95% confidence intervals of each value.

Figure 3. The relationship between the interval of vaccination and delivery (in weeks) and smoothed function of duration in generalized additive models reflecting cord anti-PT (a), anti-FHA (b) and anti-PRN (c). The increase in the time interval leads to the increase in the smoothed function thus leading to higher antibody titers. It is observed that the smooth lines were steep when the interval increased between 2 and 8 weeks, but the steepness decreased between 8 and 14 weeks for all three antibodies tested. We concluded from the graph that the best timing to maximize the cord titers was at least eight weeks prior to delivery.

Supplementary Table

Table S1. Duration of redness after Tdap vaccination during pregnancy.

Table S2. Duration of fever after Tdap vaccination during pregnancy.

Table S3. Duration of swelling after Tdap vaccination during pregnancy.

Table S4. Duration of pain after Tdap-vaccination during pregnancy.

Table S5. Comparisons of adverse events after Tdap vaccination during pregnancy in women who have or have not had prior immunization with tetanus vaccine.

Appendix

Appendix 1. Inclusion and Exclusion criteria for pregnant women in this study.

Table 1

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Figure 1

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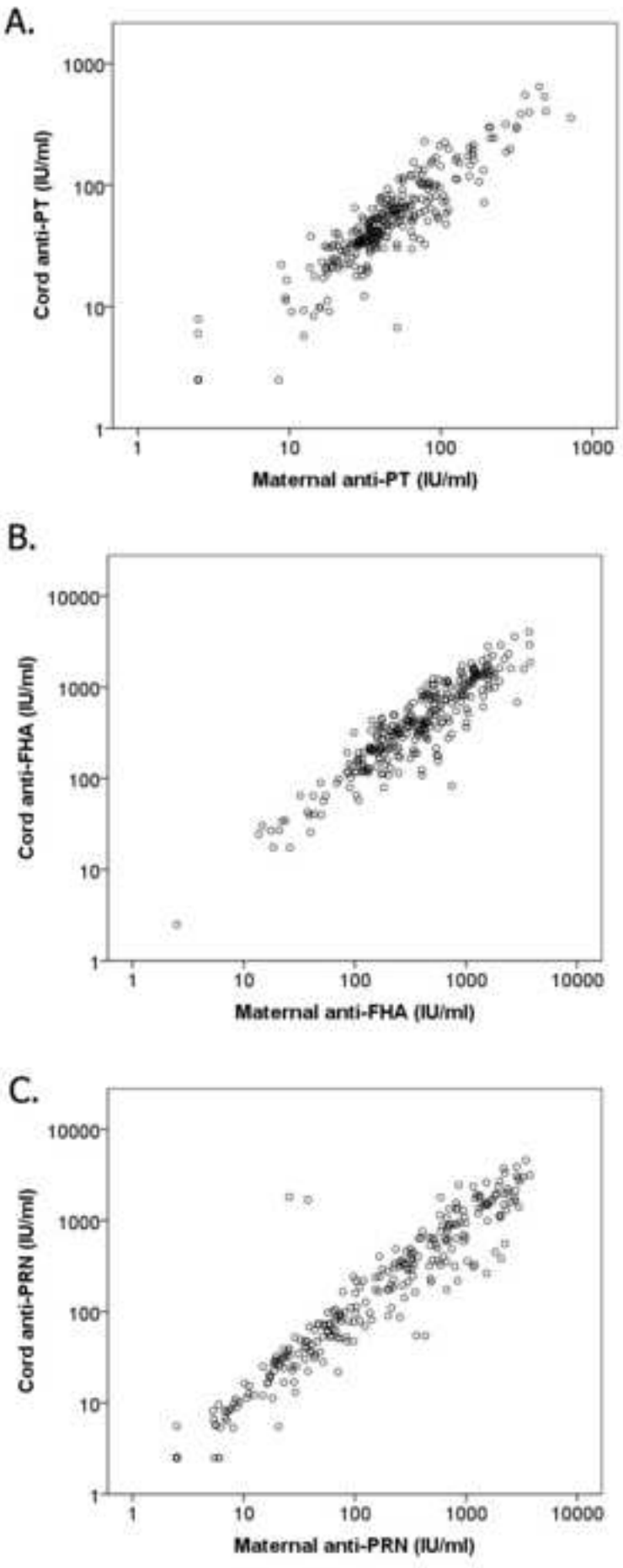

Figure 2

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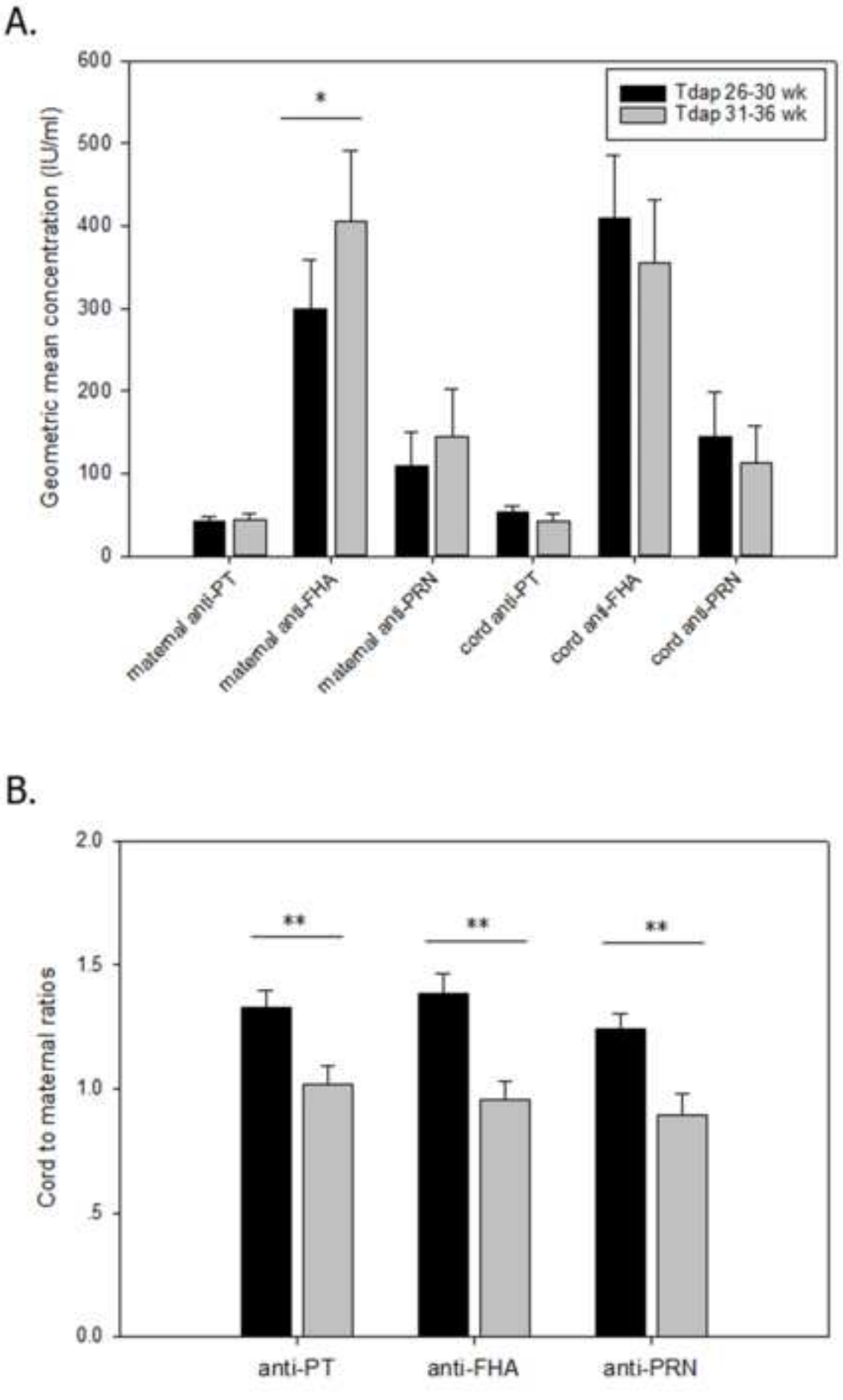

Figure 3

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