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Original article

Timing of maternal Tdap vaccination: A dynamic balance between antibody induction and placental transfer efficiency

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ABSTRACT

Background: Tetanus, diphtheria, acellular pertussis (Tdap) vaccination in pregnancy protects newborns against pertussis, but the influence of gestational age (GA) at vaccination on maternal and neonatal immune profiles remains incompletely understood. This study aimed to characterize the effect of GA at Tdap vaccination on several antibody features during pregnancy and at birth, and to assess transplacental antibody transfer.

Methods: 96 pregnant women received Tdap at different GAs between week 16 and 32 within a Belgian, prospective non-randomized controlled trial. Maternal blood was collected pre-vaccination, at multiple timepoints post-vaccination, and at delivery, alongside cord blood at birth. Tdap-specific total IgG and IgG subclasses were evaluated alongside Fc-mediated effector functions. Multivariate analyses were applied to define composite immune patterns.

Results: Post-vaccination, robust immune responses were observed across cohorts. At delivery, maternal total IgG and IgG1 against PRN, DT, and TT were higher with later vaccination, whereas other subclasses and functional responses were largely comparable. Cord blood profiles partially paralleled maternal patterns without reaching significance, and no significant effect of vaccination-to-delivery interval was detected. IgG transfer ratios generally declined with advancing GA; functional antibody transfer was largely unaffected. Multivariate analyses highlighted higher maternal antibody response profiles at delivery with later vaccination, while cord blood profiles were generally unaffected.

Conclusion: Maternal Tdap antibody response profiles at delivery are influenced by vaccination timing, while neonatal antibody profiles at birth appear largely comparable within the limits of the study. These findings support current recommendations for Tdap administration between 16 and 32 weeks of gestation and underscore the flexibility of this window for routine antenatal care.

1. Introduction

Pertussis, or whooping cough, remains a serious threat to young infants, particularly in the first months of life before completion of the primary vaccination series. Despite widespread immunization programs, pertussis continues to re-emerge with recurrent outbreaks every 2–5 years [1–3]. These outbreaks highlight waning immunity and the need for effective strategies to protect neonates too young to benefit from their own vaccination [4].

Vaccination during pregnancy with a tetanus-diphtheria-acellular pertussis (Tdap) vaccine has become the primary intervention to protect neonates against pertussis in early life. Administered during pregnancy, it boosts maternal antibody production, enabling passive protection of the newborn through Fc-mediated transplacental transfer of maternal antibodies [5]. Many countries now recommend maternal Tdap vaccination, but guidance on timing varies, from as early as 16 weeks to the late second or early third trimester of pregnancy [6–8]. This variation reflects uncertainties about the optimal timing to maximize protection. As no correlate of protection exists for pertussis, disease-specific cord blood antibody levels serve as a surrogate marker of

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neonatal protection [9,10]. Consequently, most studies assessing timing focus on antibody titers in cord blood at birth. However, findings are inconsistent, as studies variably report higher levels following second- or third-trimester vaccination, or no clear difference [8], potentially reflecting biological and methodological differences across studies and other underlying antibody characteristics that impact transplacental transfer.

Beyond antibody quantity, IgG antibodies encompass diverse biophysical and functional features that shape their protective capacity and placental transfer dynamics. Besides neutralization, Fc-mediated effector functions like complement activation and phagocytosis are critical for immune protection [11,12]. IgG subclasses differ in structure and affinity for the neonatal Fc receptor (FcRn) that mediates transplacental antibody transfer, while Fc glycosylation dynamically changes across gestation [13]. Both IgG subclass and Fc glycosylation shape Fc-receptor binding – and thus effector function – and may also influence transfer efficiency, though the role of Fc glycans in transplacental transport is debated [12,13]. Clinical factors such as parity and gestational age at birth may further affect antibody transfer [14–17].

Because dynamic immunological changes occur throughout gestation, the timing of Tdap vaccination in pregnancy may not only impact the magnitude of antibody responses but also the quality of antibodies. Understanding these dynamics is crucial for optimizing maternal immunization strategies. The present study investigates how gestational age at Tdap vaccination affects quantitative and qualitative features (IgG subclasses and Fc-mediated effector functions) of the maternal IgG antibody response during pregnancy and at birth, and how it impacts transplacental antibody transfer.

2. Materials & methods

2.1. Study design

This prospective non-randomized controlled trial was conducted in Belgium between September 2021 and October 2023 (NCT06466629). Pregnant women without a pertussis-containing vaccine in the past 5 years were recruited into three cohorts based on gestational age (GA) at the moment of first contact (inclusion and exclusion criteria listed in Supplementary Table S1). The licensed Tdap vaccine Triaxis® (Sanofi, France) was used, containing five antigens purified from *B. pertussis* (2.5 µg PT, 5 µg FHA, 3 µg PRN, 5 µg FIM2 and 5 µg FIM3), ≥2 IU diphtheria toxoid and ≥ 20 IU tetanus toxoid [18]. The vaccine was administered by the study personnel within predefined gestational windows: cohort 1 (C1) 16–18 weeks of gestation (WG) (early second trimester); cohort 2 (C2) 25–27 WG (late second trimester); cohort 3 (C3) 30–32 WG (early third trimester). No other vaccines were administered concomitantly. Blood samples were collected by the study personnel within 72 h before Tdap vaccination, 28–35 days post-vaccination, and subsequently every 4 weeks until delivery. At birth, maternal and cord blood were collected by the hospital staff (schematic representation in Supplementary Fig. S1). Blood samples were centrifuged (10 min, 1500 *g*) and serum was stored at −20 °C until analysis. Maternal demographic and obstetric data were collected at baseline and at each follow-up visit until birth. The study was approved by the Ethics Committee of the University Hospital Antwerp-University of Antwerp. As the vaccine used was a licensed product administered in accordance with the Belgian Superior Health Council guidelines for maternal immunization [19], the primary aim of this study did not include systematic safety follow-up for adverse events (AEs) or serious adverse events (SAEs) following vaccination. Nevertheless, at each study visit, parents were asked to report any AEs or SAEs, and the medical history of AEs and SAEs occurring in the participant's household was assessed.

2.2. Laboratory assays

2.2.1. Antigen-specific IgG

Total IgG antibody levels against pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), diphtheria toxin (DT) and tetanus toxin (TT) were quantified in serum using commercial ELISA kits (EUROIMMUN, United Kingdom) calibrated to WHO standards, following manufacturer instructions. Photometric measurements were acquired with a Victor Nivo (Perkin Elmer, United States) multimode plate reader. Standard curves were generated for each assay and measurements were interpolated from the calibration curve into international units per milliliter (IU/mL).

2.2.2. Antigen-bead conjugation

For the multiplex assays, fluorescent MagPlex® microspheres (Luminex, United States), activated with 50 mg/mL EDC and 50 mg/mL Sulfo-NHS (ThermoFisher Scientific Pierce, #77149 and #24520) were incubated with 6.25 µg of the following antigens: PT (List labs, #180), FHA (Sigma-Aldrich, #F5551), PRN (NAC, #NAT41666), DT (List labs, #151) and TT (MassBiologics, #LP1105P) at room temperature (RT) (2 h, orbital shaking, 700 rpm, dark).

2.2.3. Antigen-specific IgG subclasses

Tdap-specific IgG subclass levels were quantified using an *in-house* bead-based multiplex immunoassay [20], adapted for this study. Serum was incubated with antigen-coupled beads at RT (2 h, orbital shaking, 700 rpm). Captured IgG was detected with 0.65 µg/mL R-phycoerythrin-labelled detection antibody specific for each subclass, including IgG1 (Southern Biotech #9052–09), IgG2 (Southern Biotech #9070–09), IgG3 (Southern Biotech #9210–09), and IgG4 (Southern Biotech #9200–09). Antigen-antibody binding was measured as mean fluorescence intensity (MFI) on a BioPlex-200 (Bio-Rad, California, USA). For each analyte, acquisition targeted a minimum of 50 beads per sample. Samples were analyzed in single measurements and reported as MFIs. To minimize potential batch effects, samples from different cohorts were distributed across assay plates, and WHO international standard NIBSC 06/140 and blanks were included on each plate for quality control. No additional normalization or batch-correction procedures were applied. Several serum dilutions were tested, with final selections based on optimal signal detection within the linear range and aligned with the standard: 1:6000 for IgG1 (PT), 1:10000 for IgG1 (FHA, PRN, DT and TT), 1:1000 for IgG2 and 1:200 for IgG3 and IgG4.

2.2.4. Serum IgG purification

For the functional assays, IgG was purified from serum using Melon Gel resin following the manufacturer's instructions (Thermo Scientific #45208) to ensure standardized assessment of the effector functions attributable specifically to IgG.

2.2.5. Antibody-dependent complement deposition

Antibody-dependent complement deposition (ADCD) of antibodies against PT, FHA, PRN, DT and TT was measured using an *in-house* bead-based multiplex immunoassay [20], adapted for this study. Purified IgG at 1:20 dilution was incubated with Tdap-specific antigen-coupled beads (RT, 2 h, orbital shaking, 700 rpm). The captured IgG was incubated with human complement serum (Sigma #S1764) at concentration 1:50 (30 min, 37 °C). Biotinylated monoclonal C3d antibody (QuidelOrtho, #A702) and streptavidin-RPE (Agilent, # PJ31S-1) were added sequentially (30 min, RT, orbital shaking, 700 rpm). Complement deposition was determined on a BioPlex-200 (Bio-Rad, California, USA), obtaining MFIs. WHO international standard NIBSC 06/140 and blanks were included on each plate.

2.2.6. Antibody-dependent cellular phagocytosis

Antibody-dependent cellular phagocytosis (ADCP) of PT-specific antibodies was measured with an *in-house* bead-based immunoassay

Table 1
Demographic and obstetric characteristics of the selected study participants. Categorical data were compared with Chi-square or Fisher's Exact tests depending on the expected frequency of values. Continuous data were compared with Kruskal-Wallis, and pairwise Mann-Whitney U tests when appropriate (KW $p < 0.05$). Significant comparisons are highlighted in bold. Abbreviations: GA, gestational age; IQR, interquartile range; N, number; Tdap, tetanus-diphtheria-acellular pertussis.

Parameter	Cohort 1	Cohort 2	Cohort 3	*p*-value
	N = 32	N = 32	*N* = 30	
Mother				
Median age at inclusion, years (IQR)	31.4 (29.5–35.2)	32.1 (29.9–34.0)	31.6 (29.0–34.3)	0.778
Ethnicity, N (%)				
Caucasian	32 (100%)	32 (100%)	29 (96.7%)	0.319
Non-Caucasian	0 (0%)	0 (0%)	1 (3.3%)	
Parity, N (%)				
Uniparous	27 (84.4%)	27 (84.4%)	25 (83.3%)	0.992
Multiparous	5 (15.6%)	5 (15.6%)	5 (16.7%)	
Median GA at Tdap vaccination, weeks (IQR)	17.1 (16.5–17.6)	26.0 (25.4–26.7)	30.8 (30.4–31.7)	<0.001
Influenza vaccination during pregnancy, N (%)				
Yes	15 (46.9%)	22 (68.8%)	17 (56.7%)	0.208
No	17 (53.1%)	10 (31.3%)	13 (43.3%)	
COVID-19 vaccination during pregnancy, N (%)				
Yes	19 (59.4%)	25 (78.1%)	22 (73.3%)	0.235
No	13 (40.6%)	7 (21.9%)	8 (26.7%)	
Delivery				
Median GA at delivery, weeks (IQR)	39.5 (38.5–40.4)	39.8 (38.8–40.3)	39.4 (38.9–40.1)	0.789
Median interval Tdap vaccination and delivery, weeks (IQR)	22.3 (21.6–23.3)	13.6 (12.9–14.6)	8.6 (7.3–9.6)	<0.001
Term and preterm births, N (%)				
Term	29 (90.6%)	32 (100.0%)	28 (93.3%)	0.275
Preterm	3 (9.4%)	0 (0%)	2 (6.7%)	
Mode of delivery, N (%)				
Vaginal	22 (68.8%)	26 (81.3%)	21 (70.0%)	0.463
Caesarean	10 (31.3%)	6 (18.8%)	9 (30.0%)	
Induction of labor, N (%)				
Yes	10 (31.3%)	8 (25.0%)	7 (23.3%)	0.756
No	22 (68.8%	24 (75.0%)	23 (76.7%)	
Infant				
Sex, N (%)				
Male	16 (50.0%)	15 (46.9%)	15 (50.0%)	0.96
Female	16 (50.0%)	17 (53.1%)	15 (50.0%)	
Median birth weight, kg (IQR)	3.4 (3.1–3.6)	3.5 (3.2–3.7)	3.5 (3.2–3.7)	0.209
Median birth length, cm (IQR)	50.0 (49.0–51.0)	50.0 (49.0–52.0)	50.8 (48.0–52.0)	0.242

[3], adapted for this study. Biotinylated antigen purified using Melon Gel resin following the manufacturer's instructions (Thermo Scientific #45206) was incubated with neutravidin beads (Invitrogen, F8776) (2 h, 37 °C, 5% CO_2). The antigen-coated beads were incubated with the purified serum IgG at 1:10 dilution (2 h, 37 °C, 5% CO_2.). The antigen-antibody complexes were incubated overnight with cultured THP-1 cells (ATCC, TIB-202) (100,000 cells per well, 37 °C, 5% CO_2). Cells were fixed, and phagocytic activity was measured using flow cytometry. Results were analyzed using FlowJo software version 10.10.0 (BD) by gating on THP-1 cells and expressed as:

$$\text{Phagocytic score (PS)} = \frac{\%\text{bead}^+ \text{ THP1 cells x geometric mean MFI bead}^+ \text{ THP1 cells}}{10^4}$$

2.2.7. Antibody-dependent neutrophil phagocytosis

Antibody-dependent neutrophil phagocytosis (ADNP) of PT-specific antibodies was measured with an *in-house* bead-based immunoassay [4], adapted for this study. Purified biotinylated beads were incubated with purified serum IgG at 1:30 dilution (2 h, 37 °C, 5% CO_2). The antigen-antibody complexes were incubated with neutrophils isolated from fresh blood of a single healthy donor (100,000 cells per well, 2 h, 37 °C, 5% CO_2) and within a single experimental run to minimize variability attributable to differences in neutrophil function between donors. Cells were stained with CD66b-Pacific Blue antibody (Biolegend, #305112). After fixation, phagocytic activity was measured by flow cytometry. Results were analyzed using FlowJo software version 10.10.0 (BD) by gating on CD66b + neutrophils and expressed as:

$$\text{Phagocytic score (PS)} = \frac{\%\text{bead}^+ \text{ neutrophils x geometric mean MFI bead}^+ \text{ neutrophils}}{10^4}$$

2.3. Statistical analysis

Cohort differences in demographic and obstetric categorical variables were tested with Chi-square or Fisher's Exact tests; continuous variables with Kruskal-Wallis tests, followed by pairwise Mann-Whitney *U* tests when significant (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The transplacental transport ratio (TTR) was calculated as cord:maternal serum antibody ratio and corrected with the ROUT method to reduce outlier bias (Q = 0.1%). Correction for multiple testing was not applied given the exploratory nature of the study. Principal component analysis (PCA) was performed at three timepoints (28–35 days post-vaccination, delivery maternal blood, delivery cord blood) including all tested features, to identify potential patterns or clusters in the complex dataset. Components with eigenvalues >1 were considered informative according to the Kaiser criterion, as they explain more variance than an individual original variable. All analyses were performed using SPSS Statistics 29.0.2.0 and GraphPad Prism, version 10.6.0.

3. Results

3.1. Study population

All participants were recruited in Belgium between September 2021 and October 2023 and delivered between January 2022 and April 2024. Cohort assignment was dependent on the GA at the moment of first

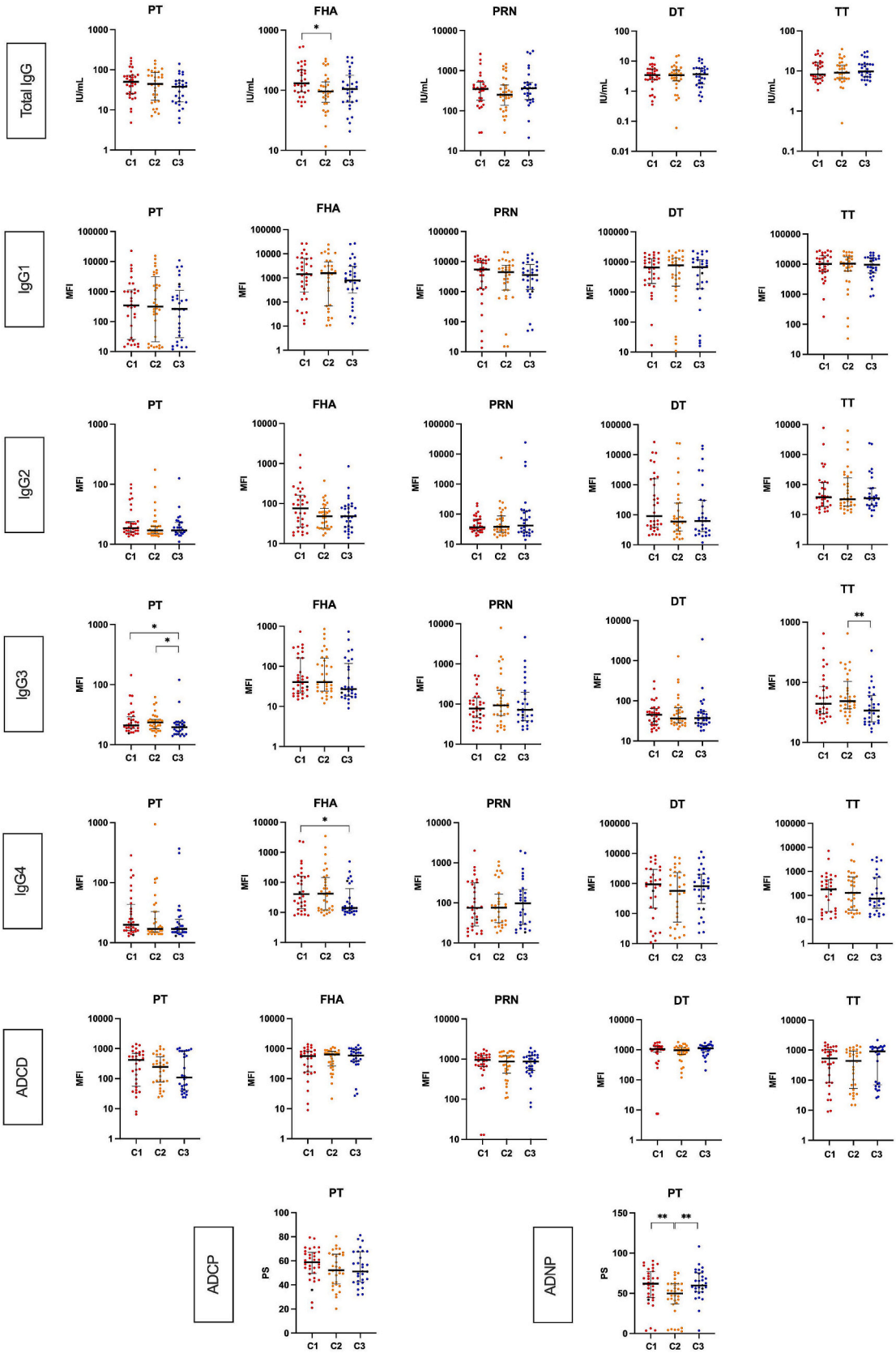

Fig. 1. Antigen-specific IgG levels of total and subclass IgG, antibody-dependent complement deposition (ADCD) activity, antibody-dependent cellular phagocytosis (ADCP) activity and antibody-dependent neutrophil phagocytosis (ADNP) activity at 28–35 days post-vaccination, per cohort. Individual data and median with interquartile ranges are plotted. Statistical significance between cohorts tested with Kruskal-Wallis, and pairwise Mann-Whitney *U* tests when appropriate (KW $p < 0.05$) (red = C1; yellow = C2; blue = C3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contact with the study team. Each cohort initially included 32 participants. In cohort 3 (C3), one participant dropped out due to pregnancy complications and another was excluded for immunosuppressive medication use, leaving 30 participants in C3 and 94 participants overall. Demographic and obstetric characteristics are presented in Table 1. No significant differences were observed between cohorts, except for GA at Tdap vaccination and the vaccination-to-delivery interval. For both, all pairwise comparisons were significantly different ($p < 0.001$), as expected from the study design. No vaccine-related SAEs were reported by parents during the study.

In total, 552 serum samples were collected across all cohorts and visits, with only 12 scheduled samples missing (mainly delivery samples due to uninformed hospital staff). Information on collection completeness is provided in Supplementary Table S2.

3.2. *Vaccine-induced IgG profile during pregnancy*

Tdap-specific total and subclass IgG levels, Tdap-specific ADCD, and PT-specific ADCP and ADNP responses at 28–35 days post-vaccination are depicted per cohort in Fig. 1. Overall, responses were comparable across cohorts, with a limited number of significant differences observed: anti-FHA total IgG was lower in C2 than in C1, anti-PT IgG3 was lower in C3 than in C1 and C2, anti-TT IgG3 was lower in C3 versus C2, anti-FHA IgG4 was lower in C3 than in C1, and PT-specific ADNP responses were lower in C2 than in C1 and C3. IgG3 differences were already observed prior to vaccination (Supplementary Fig. S2). Additional significant baseline differences included higher anti-TT IgG1 in C3 versus C1 and lower anti-DT IgG3 in C3 versus C1 and C2. Wilcoxon signed-rank tests comparing pre-vaccination and peak levels confirmed significant increases across all cohorts, antigens and immunological

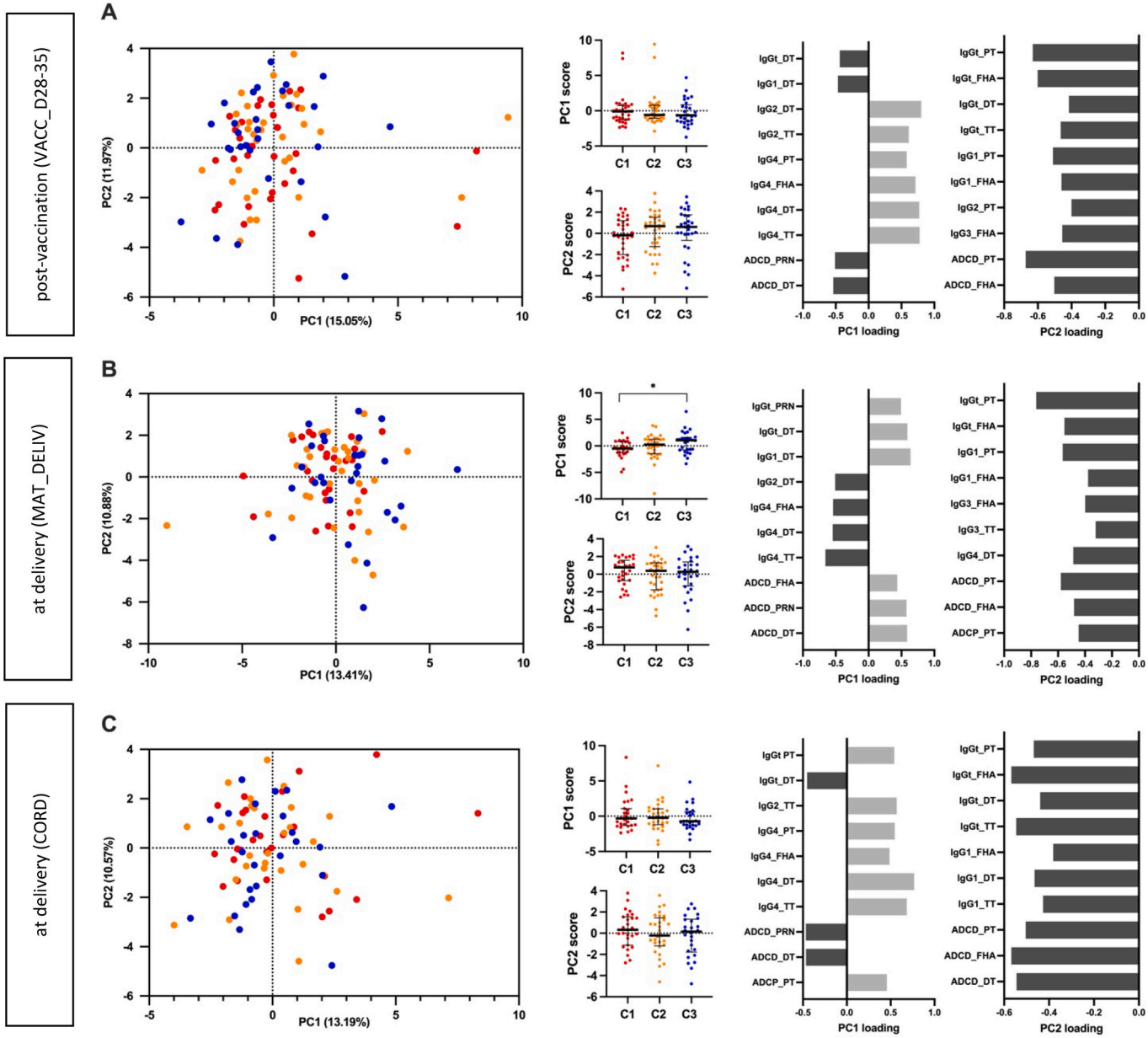

Fig. 2. Principal component analyses of antibody profiles in A) maternal blood 28–35 days after Tdap vaccination, B) maternal blood at delivery, and C) cord blood at delivery. Scatter plots of PCAs (left panels); PC1 and PC2 scores per cohort, with individual data and median with interquartile ranges plotted, and statistical significance between cohorts tested with Kruskal-Wallis, and pairwise Mann-Whitney U tests when appropriate (KW $p < 0.05$) (middle panels); and the 10 immunological features with the strongest loadings in PC1 and PC2 (right panels). (red = C1; yellow = C2; blue = C3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

features (all $p < 0.001$). The antigen- and feature-specific antibody kinetics set by the time interval between vaccination and visit per cohort are presented in Supplementary Fig. S3. After the initial rise, most IgG responses gradually declined across cohorts until delivery although some antigen- and subclass-specific responses showed little change over time. Antigen-specific total IgG geometric mean concentrations per antigen, cohort and visit are presented in Supplementary Table S3.

To explore overall patterns, all antibody features at 28–35 days post-vaccination were integrated into a PCA (Fig. 2A). Eleven components had eigenvalues >1 (not shown), indicating that multiple independent dimensions contributed to the immune response profile. The first two principal components (PC1 and PC2) were visualized, explaining 27.0% of the total variance, while the remaining nine components accounted for an additional 45.4%. No cohort clustering was observed, and PC scores did not differ significantly between cohorts. PC1 was predominantly driven by antigen-specific IgG2 and IgG4 responses (positive loadings) and by antigen-specific ADCD, total IgG and IgG1 responses (negative loadings).

3.3. IgG profile at delivery

In maternal blood at delivery, total IgG anti-PT and -FHA were comparable across cohorts, whereas IgG against PRN, DT and TT increased with advancing GA at vaccination. Differences reached significance for anti-PRN and -TT IgG in C3 versus C1 and C2, and for anti-DT IgG in C3 versus C1 (Fig. 3). IgG1 results were partially similar, with comparable levels against PT, FHA and PRN across cohorts, but higher anti-DT and -TT with advancing GA at vaccination, significant for anti-TT IgG1 in C3 versus C1 and C2. IgG2, IgG3 and IgG4 levels were generally low and comparable across cohorts, except for significantly higher anti-PRN IgG2 in C3 versus C1 and significantly lower anti-PT IgG3 in C3 versus C2. Fc-mediated effector functions were broadly comparable. PCA of maternal delivery samples identified 12 components with eigenvalues >1, indicating that multiple dimensions contributed to variation in the antibody response profile (not shown). PC1 and PC2 accounted for 24.3% of the total variance and were used for visualization (Fig. 2B), indicating significantly higher PC1 scores in C3 compared with C1, mainly driven by IgG/IgG1 differences and ADCD responses against DT and PRN (positive loadings), and in contrast with IgG2/IgG4 (negative loadings). The remaining ten components accounted for 51.1% of the variance.

In cord blood, total IgG against PT and FHA was largely comparable across cohorts (Fig. 4). IgG against PRN, DT and TT tended to be higher with increasing GA at vaccination but without reaching statistical significance. IgG1 against PT, FHA and PRN was comparable across cohorts, whereas a trend for higher levels with increasing GA at vaccination was observed for IgG1 against DT and TT, although only significant for anti-TT in C3 versus C2. IgG2, IgG3 and IgG4 levels in cord blood were generally low and mostly comparable across cohorts, except for significantly lower anti-PT IgG3 in C3 versus C1 and C2 and significantly lower anti-FHA IgG4 in C3 versus C1. Comparable effector function activity was measured except for lower anti-PT ADNP in cord blood in C1 versus C2. PCA in cord blood identified 12 components with eigenvalues >1, indicating that multiple dimensions contributed to variation in the antibody response profile (not shown). PC1 and PC2 explained 23.8% of the variance and were visualized for examination of overall response patterns (Fig. 2C). No cohort clustering was observed and PC scores did not differ significantly between cohorts. PC1 was driven predominantly by IgG4/IgG2 responses for all antigens and particularly against DT, TT, and PT (positive loadings). The remaining ten components accounted for 50.7% of the variance.

Maternal and cord blood antibody levels correlated strongly at delivery (Spearman correlation coefficient $r > 0.7$ for most features and antigens; Supplementary Table S4). Only ADNP levels in C2 and C3 were not significantly correlated. Correlations were generally strongest for total IgG, IgG2, IgG4 and ADCD responses, whereas comparatively weaker correlations were observed for TT-specific IgG1 and IgG3 responses. Spearman correlation analyses between cord blood antibody levels and vaccination-to-delivery interval showed weak associations across all features and antigens (Spearman $r = -0.23$ to 0.24; Supplementary Table S5), none of which were statistically significant.

3.4. IgG transplacental transfer

TTRs of total IgG consistently declined with increasing GA at vaccination, reaching significance for FHA, PRN and TT in C3 versus C1. Subclass-specific TTRs showed similar but antigen-dependent differences including significantly lower anti-TT IgG1 in C3 versus C1, lower anti-FHA and anti-TT IgG2 in C3 versus C1, lower anti-PRN IgG2 in C2 versus C1, lower anti-PT IgG3 in C3 versus C1, lower anti-FHA IgG3 in C2 versus C1, and lower anti-FHA IgG4 in C3 versus C1. No significant trends were observed for functional antibody features across cohorts (Fig. 5).

4. Discussion

The optimal timing of Tdap vaccination in pregnancy remains debated. In this study, we assessed how Tdap vaccination at different gestational ages affects maternal antibody responses and their transfer to the neonate. Although maternal antibody profiles at delivery were generally higher with increasing GA at vaccination, antibody profiles 28–35 days post-vaccination and in cord blood at delivery were largely unaffected by timing of vaccination, with comparable baseline characteristics across cohorts minimizing confounding. The few pre-vaccination antibody profile differences were partially reflected at 28–35 days post-vaccination.

Maternal Tdap vaccination at three gestational age windows yielded comparable maternal antibody profiles 28–35 days after immunization, as supported by univariate and integrative analyses. Peak IgG levels were significantly higher than pre-vaccination across cohorts and features and antigens, indicating robust immune responses to Tdap vaccination irrespective of GA at vaccination. After the initial rise, most IgG responses gradually declined across cohorts until delivery. These results align with prior similarly designed studies, though those typically sampled only once post-vaccination in pregnancy and measured titers only [21–23].

At delivery, antigen-specific maternal total IgG was higher with later GA at vaccination, consistent with less time for antibody waning. A partially similar pattern was seen for IgG1, while other subclasses remained generally low and comparable across cohorts. Fc-mediated effector functions were also broadly comparable. Integrative analysis confirmed stronger composite responses of specific features with later vaccination, largely consistent with univariate findings and suggesting a modestly less waned antibody profile in the mother at delivery following later GA at vaccination.

Cord blood IgG levels partially mirrored maternal profiles but did not differ significantly between cohorts, although the study was not powered to formally assess equivalence between groups. Subclass and functional responses were broadly comparable across cohorts and integrative analysis showed no cohort clustering, indicating a largely comparable neonatal antibody profile across cohorts. Regression analyses of cord blood IgG levels against vaccination-to-delivery interval revealed no significant correlations, suggesting no strong time-dependent effect of vaccination on cord blood antibody levels. While some previous studies reported positive associations [24,25], our findings align with other reports showing no significant association [26–29]. Overall, our data suggest that maternal antibody profiles at delivery vary with GA, but neonatal antibody levels remain largely unaffected.

Maternal-cord differences at delivery were reflected in the TTR. Total IgG transfer declined with increasing GA at vaccination, aligning with prior studies reporting lower pertussis-specific IgG transfer ratios with later vaccination [30,31], although those studies examined a

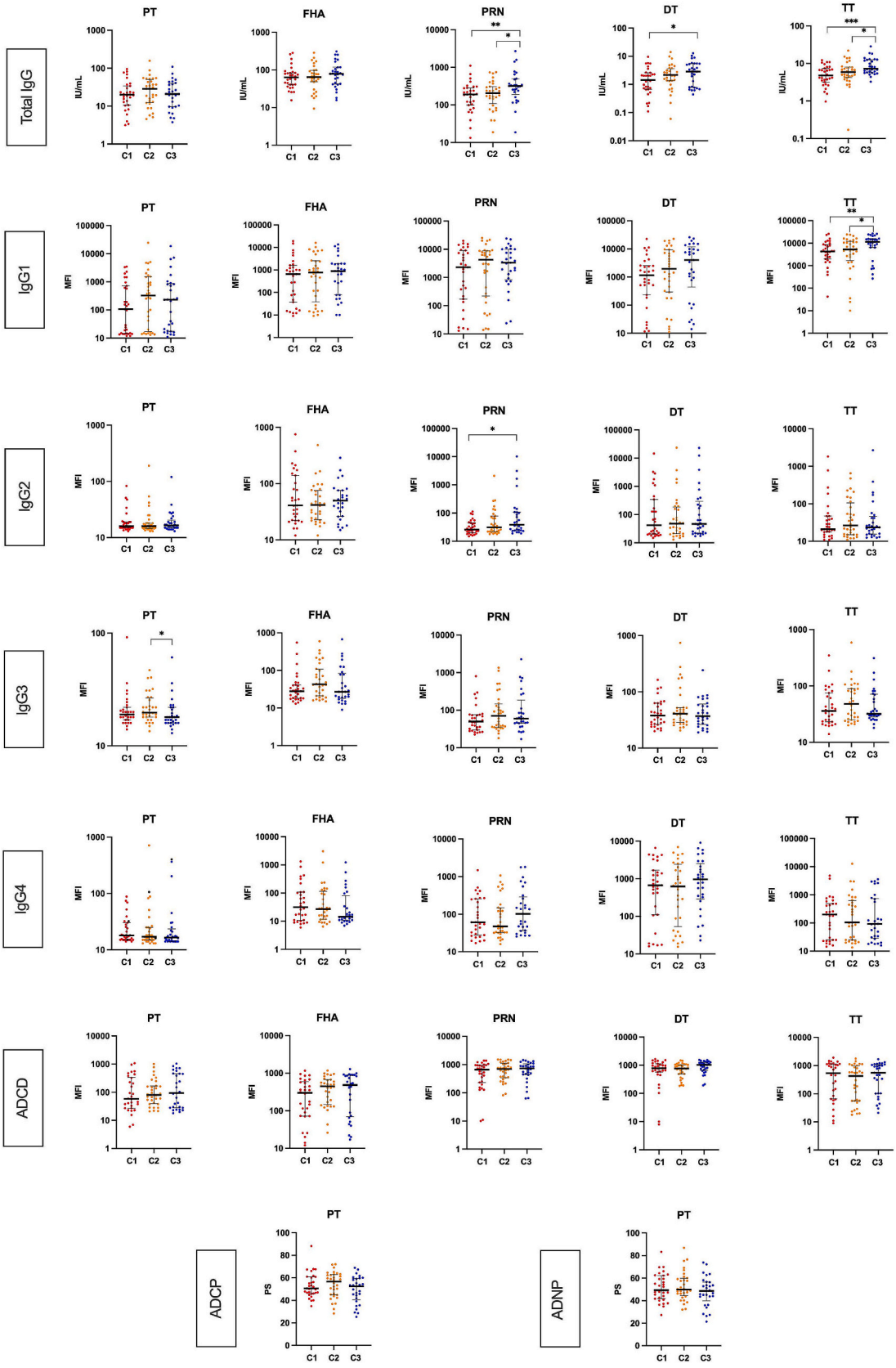

Fig. 3. Antigen-specific IgG levels of total and subclass IgG, antibody-dependent complement deposition (ADCD) activity, antibody-dependent cellular phagocytosis (ADCP) activity and antibody-dependent neutrophil phagocytosis (ADNP) activity in maternal blood at delivery, per cohort. Individual data and median with interquartile ranges are plotted. Statistical significance between cohorts was tested with Kruskal-Wallis, and pairwise Mann-Whitney U tests when appropriate (KW $p < 0.05$). (red = C1; yellow = C2; blue = C3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

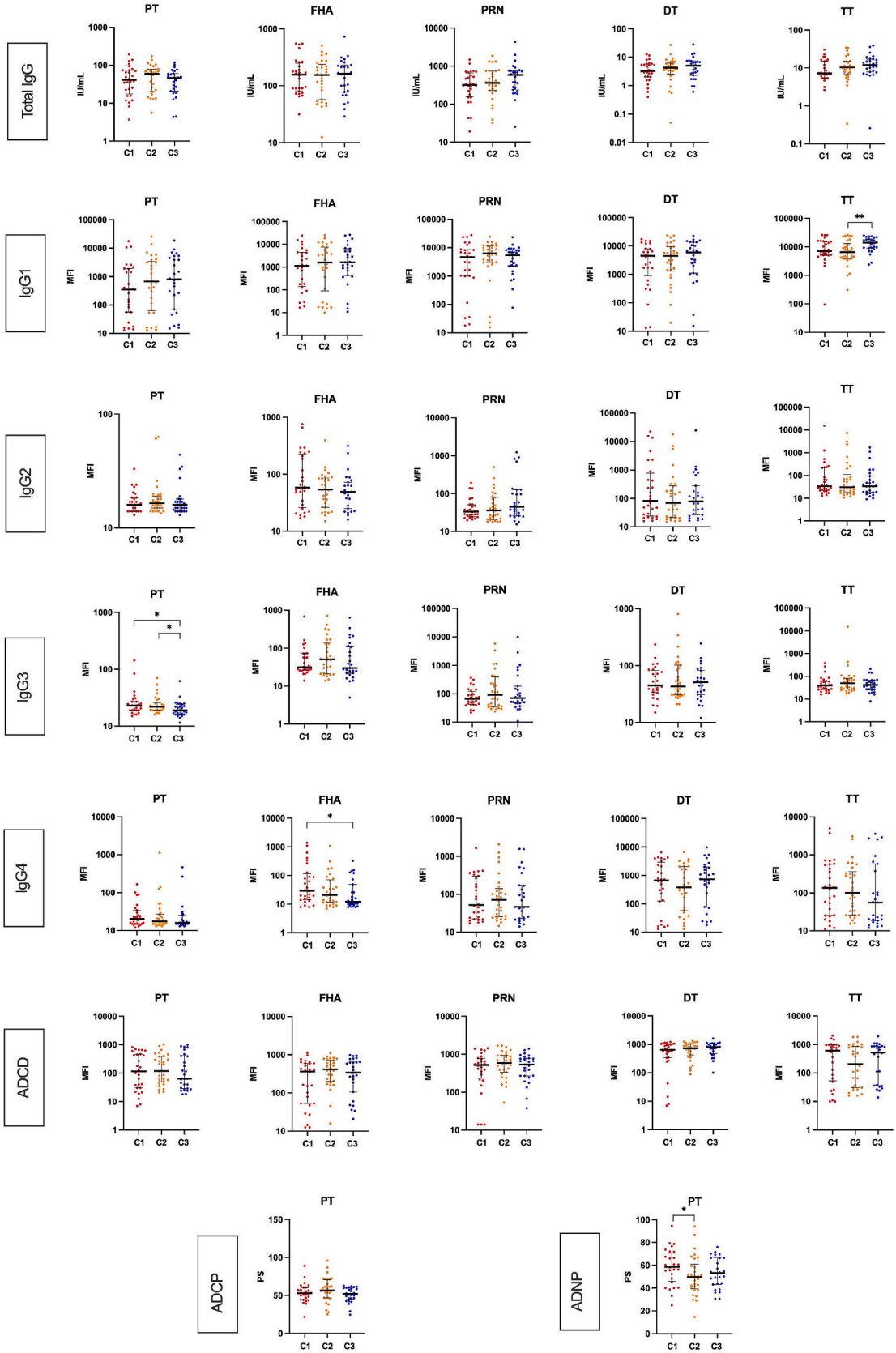

Fig. 4. Antigen-specific IgG levels of total and subclass IgG, antibody-dependent complement deposition (ADCD) activity, antibody-dependent cellular phagocytosis (ADCP) activity and antibody-dependent neutrophil phagocytosis (ADNP) activity in cord blood at delivery, per cohort. Individual data and median with interquartile ranges are plotted. Statistical significance between cohorts was tested with Kruskal-Wallis, and pairwise Mann-Whitney U tests when appropriate (KW $p < 0.05$). (red = C1; yellow = C2; blue = C3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

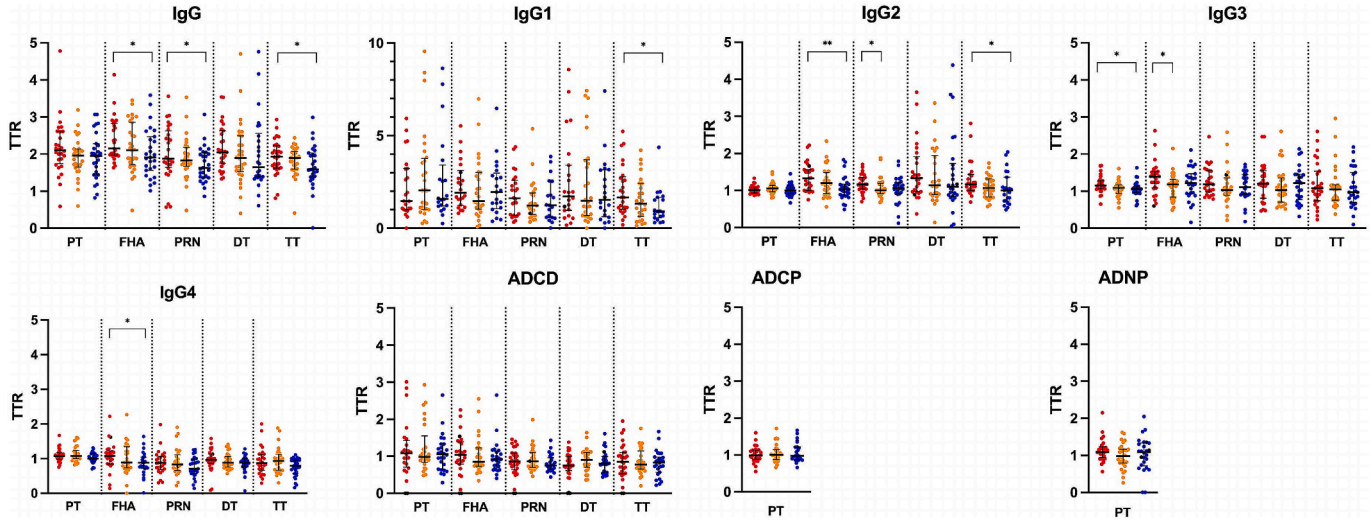

Fig. 5. Transplacental transfer ratios (TTRs) of antigen-specific total IgG, IgG subclasses, antibody-dependent complement deposition (ADCD) activity, antibody-dependent cellular phagocytosis (ADCP) activity and antibody-dependent neutrophil phagocytosis (ADNP) activity, per cohort. Individual data and median with interquartile ranges are plotted. Statistical significance between cohorts was tested with Kruskal-Wallis, and pairwise Mann-Whitney U tests when appropriate (KW $p < 0.05$). (red = C1; yellow = C2; blue = C3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

narrower gestational window and focused exclusively on total IgG. Subclass-specific, antigen-dependent patterns were biologically plausible: IgG1 transfer was favored (median TTR of 1.3–1.7), over IgG2/IgG3 (median TTR of 1.0–1.2) and IgG4 (median TTR of 0.8–1.1), consistent with IgG1 as the most efficiently transferred subclass, although still debated [32]. Despite lower IgG TTRs with increasing GA at vaccination, functional antibody transfer was largely unaffected across cohorts. Importantly, these TTR findings should be interpreted with caution, as the formula does not account for maternal antibody kinetics throughout pregnancy and increasing transplacental transfer capacity with advancing gestation [33,34].

Overall, our findings highlight a critical balance between maternal antibody generation and placental transfer efficiency in shaping neonatal antibody levels. Later vaccination yields higher maternal antibody concentrations near delivery, but less transfer time; earlier vaccination allows longer transfer but coincides with lower maternal titers during peak transfer [33,34]. The absence of strong correlations between vaccination-to-delivery interval and cord blood levels may reflect the complex interplay between maternal antibody kinetics and placental transport mechanisms, which are not linearly time-dependent. Our findings align with a similarly designed study reporting comparable cord blood anti-PT and -PRN IgG across vaccination windows [23] and extend that work by analyzing a broader range of immunological features during pregnancy and at delivery.

Hence, limitations should be acknowledged. First, sample size calculation was challenging given the limited availability of longitudinal, multi-dimensional immune response data in pregnancy and the exploratory character of the study. Therefore, the sample size was based on previously conducted immunogenicity studies in pregnant women [29,35–37] and feasibility assessment. Given the exploratory nature of the study, the absence of statistically significant differences should be interpreted with caution. Second, the study was non-randomized and – although several demographic factors were assessed – unmeasured confounding factors may have influenced the results. A comprehensive evaluation of the influence of clinical and demographic characteristics was beyond the scope of the present analysis and warrants further investigation. Third, the study population may not fully represent pregnant populations across diverse geographic, ethnic, or socioeconomic contexts, limiting generalizability. Fourth, while detailed immunological profiling was performed, clinical endpoints were not assessed as no validated correlate of protection for pertussis exists, leaving the relevance of observed differences uncertain. The cord blood antibody profile was used as a proxy for neonatal immunity, which may not fully capture postnatal immunity. In addition, samples were collected over a period exceeding two years and stored at −20 °C until analysis. Although storage at −80 °C is generally preferred for long-term antibody preservation, all cohorts were recruited concurrently and samples were processed and analyzed under standardized conditions. Consequently, any effect of storage duration on antibody measurements is expected to be non-differential across cohorts, although it cannot be completely excluded. Finally, only a limited set of effector functions was assessed. Additional effector functions and other features such as Fc glycosylation, binding avidity, and Fc receptor affinity, which may modulate transplacental transfer efficiency, were not evaluated, limiting a full understanding of the protective capacity of the antibody responses [38,39]. Further research should assess how these features contribute to protection and how Tdap timing affects cell-mediated immunity, infant immune outcomes, and breastmilk antibodies.

In conclusion, Tdap vaccination in pregnancy elicits robust antibody responses irrespective of vaccination timing. At delivery, maternal antibody profiles are influenced by timing of Tdap vaccination, but neonatal cord blood antibody profiles remain largely comparable. These findings support Tdap vaccination between 16 and 32 weeks of gestation and reinforce flexibility for timing of vaccine administration in antenatal care.

5. Summary

Maternal Tdap in pregnancy protects infants in early life against pertussis. Our data suggest that while timing of vaccination influences the maternal antibody profile at delivery, the neonatal antibody profile is largely comparable, supporting flexibility of vaccine administration within 16 to 32 weeks of gestation.

CRediT authorship contribution statement

Louise De Weerdt: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Anaïs Thiriard:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation.

Inès Vu Duc: Data curation. **Nina Reviya:** Data curation. **Pierre Van Damme:** Writing – review & editing, Resources, Funding acquisition. **Niel Hens:** Formal analysis. **Arnaud Marchant:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Kirsten Maertens:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2026.128953.

Data availability

Data will be made available on request.

References

[1] Cherry JD. The history of pertussis (whooping cough); 1906–2015: facts, myths, and misconceptions. Curr Epidemiol Rep 2015;2(2):120–30.
[2] World Health Organization (WHO). Pertussis reported cases and incidence [Internet]. Available from: https://immunizationdata.who.int/global/wiise-detail-page/pertussis-reported-cases-and-incidence; 2025.
[3] Kang HM, Lee TJ, Park SE, Choi SH. Pertussis in the post-COVID-19 era: resurgence, diagnosis, and management. Infect Chemother 2025;57(1):13–30.
[4] Domenech de Cellès M, Rohani P. Pertussis vaccines, epidemiology and evolution. Nat Rev Microbiol [Internet] 2024;22(11):722–35. Available from: doi:https://doi.org/10.1038/s41579-024-01064-8.
[5] World Health Organization. Pertussis vaccines: WHO position paper, August 2015 - recommendations. Vaccine [Internet]. 2016;34(12):1423–5. Available from: doi:https://doi.org/10.1016/j.vaccine.2015.10.136.
[6] UK Government. Guidance vaccination against pertussis (whooping cough) for pregnant women [Internet]. 2024 [cited 2025 Jul 20]. Available from: https://www.gov.uk/government/publications/vaccination-against-pertussis-whooping-cough-for-pregnant-women.
[7] U.S. Government Department of Health and Human Services. Vaccines for Pregnant Women [Internet]. 2025 [cited 2025 Jul 20]. Available from: https://www.hhs.gov/immunization/who-and-when/pregnant/index.html.
[8] De Weerdt L, Herzog SA, Van Damme P, Maertens K. Timing of pertussis vaccination during pregnancy : evidence and implementation – a systematic review. Vaccine 2024;42(21):126152. https://doi.org/10.1016/j.vaccine.2024.07.053.
[9] Barug D, Pronk I, van Houten MA, Versteegh FGA, Knol MJ, van de Kassteele J, et al. Maternal pertussis vaccination and its effects on the immune response of infants aged up to 12 months in the Netherlands: an open-label, parallel, randomised controlled trial. Lancet Infect Dis [Internet] 2019;19(4):392–401. Available from: doi:https://doi.org/10.1016/S1473-3099(18)30717-5.
[10] Callender M, Harvill ET. Maternal vaccination: shaping the neonatal response to pertussis. Front Immunol 2023;July:1–9.
[11] Nimmerjahn F, Ravetch JV. Fcγ receptors as regulators of immune responses. Nat Rev Immunol 2008;8(1):34–47.
[12] Lu LL, Suscovich TJ, Fortune SM, Alter G. Beyond binding: antibody effector functions in infectious diseases. Nat Rev Immunol [Internet] 2018;18(1):46–61. doi:https://doi.org/10.1038/nri.2017.106.
[13] Rice TF, Holder B, Kampmann B. Antibody glycosylation in pregnancy and in newborns: biological roles and implications. Curr Opin Infect Dis 2020;33(3):225–30.
[14] Vidarsson G, Dekkers G, Rispens T, Klinman D. IgG subclasses and allotypes: from structure to effector functions. Front Immunol [Internet]. 2014;5(520):1–17. Available from: www.frontiersin.org.
[15] Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O. Protecting the newborn and young infant from infectious diseases: lessons from immune ontogeny. Immunity [Internet] 2017;46:350–63. Available from: doi:https://doi.org/10.1016/j.immuni.2017.03.009.
[16] Borghi S, Bournazos S, Thulin NK, Li C, Gajewski A, Sherwood RW, et al. FcRn, but not FcγRs, drives maternal-fetal transplacental transport of human IgG antibodies. Proc Natl Acad Sci [Internet] 2020;117(23):12943–12951. Available from: https://doi.org/10.1073/pnas.2004325117.
[17] De Weerdt L, Thiriard A, Leuridan E, Marchant A, Maertens K. Immunogenicity at delivery after Tdap vaccination in successive pregnancies. Front Immunol [Internet] 2024;15:1–9. Available from: https://doi.org/10.3389/fimmu.2024.1360201/full.
[18] Health Products Regulatory Authority. Summary of product characteristics Triaxis. 2021.
[19] Hoge Gezondheidsraad. Immunisatie tijdens de zwangerschap: Belgische richtlijnen [Internet]. 2020. Available from: https://www.health.belgium.be/sites/default/files/uploads/fields/fpshealth_theme_file/20201106_hgr-8754_immunisatie_tijdens_de_zwangerschap_vweb.pdf.
[20] Thiriard A, Meyer B, Eberhardt CS, Loevy N, Grazioli S, Adouan W, et al. Antibody response in children with multisystem inflammatory syndrome related to COVID-19 (MIS-C) compared to children with uncomplicated COVID-19. Front Immunol 2023;14(March):1–13.
[21] Munoz FM, Bond NH, Maccato M, Pinell P, Hammill HA, Swamy GK, et al. Safety and immunogenicity of tetanus diphtheria and acellular pertussis (Tdap) immunization during pregnancy in mothers and infants: a randomized clinical trial. JAMA [Internet] 2014;311(17):1760–9. Available from: www.clinicaltrials.gov.
[22] Healy CM, Rench MA, Swaim LS, Timmins A, Vyas A, Sangi-Haghpeykar H, et al. Kinetics of maternal pertussis-specific antibodies in infants of mothers vaccinated with tetanus, diphtheria and acellular pertussis (Tdap) during pregnancy. Vaccine [Internet]. 2020;38(37):5955–61. Available from: doi:https://doi.org/10.1016/j.vaccine.2020.06.050.
[23] Calvert A, Amirthalingam G, Andrews N, Basude S, Coleman M, Cuthbertson H, et al. Optimising the timing of whooping cough immunisation in mums (OpTIMUM) through investigating pertussis vaccination in pregnancy: an open-label, equivalence, randomised controlled trial. The Lancet Microbe 2023;4(5):e300–8.
[24] Vaz-de-Lima LRA, Sato HK, Fernandes EG, Sato APS, Pawloski LC, Tondella ML, et al. Association between the timing of maternal vaccination and newborns' anti-pertussis toxin antibody levels. Vaccine [Internet]. 2019;37(36):5474–80. Available from: https://www.sciencedirect.com/science/article/pii/S0264410X19305675?via%3Dihub.
[25] Ozdil M, Sonmez C, Tugberk Bakar M, Davutoglu EA, Madazli R, Kilic S, et al. Pertussis antibody levels in infants and their mothers receiving combined tetanus-diphtheria toxoid and acellular pertussis vaccine during pregnancy in Turkey. Eur J Obstet Gynecol Reprod Biol [Internet] 2021;265:2012–216. Available from: https://www.sciencedirect.com/science/article/pii/S0301211521004449?via%3Dihub.
[26] Hardy-Fairbanks AJ, Pan SJ, Decker MD, Johnson DR, Greenberg DP, Kirkland KB, et al. Immune responses in infants whose mothers received tdap vaccine during pregnancy. Pediatr Infect Dis J 2013;32(11):1257–60.
[27] Vilajeliu A, Goncé A, López M, Costa J, Rocamora L, Ríos J, et al. Combined tetanus-diphtheria and pertussis vaccine during pregnancy: transfer of maternal pertussis antibodies to the newborn. Vaccine 2015;33(8):1056–62.
[28] Abraham C, Pichichero M, Eisenberg J, Singh S. Third-trimester maternal vaccination against pertussis and pertussis antibody concentrations. Obstet Gynecol 2018;131(2):364–9.
[29] Fallo AA, Neyro SE, Manonelles G V, Lara C, Hozbor D, Zintgraff J, et al. Prevalence of pertussis antibodies in maternal blood, cord serum, and infants from mothers with and those without Tdap booster vaccination during pregnancy in Argentina. J Pediatric Infect Dis Soc [Internet] 2018;(7):11–8. Available from: https://academic.oup.com/jpids/article/7/1/11/2768892.
[30] Wanlapakorn N, Maertens K, Chaithongwongwatthana S, Srimuan D, Suratannon N, Vongpunsawad S, et al. Assessing the reactogenicity of Tdap vaccine administered during pregnancy and antibodies to *Bordetella pertussis* antigens in maternal and cord sera of Thai women. Vaccine [Internet]. 2018;36:1453–9. Available from: doi:https://doi.org/10.1016/j.vaccine.2018.01.059.
[31] Lima L, Molina M da GF, Pereira BS, Nadaf MLA, Nadaf MIV, Takano OA, et al. Acquisition of specific antibodies and their influence on cell-mediated immune response in neonatal cord blood after maternal pertussis vaccination during pregnancy. Vaccine [Internet]. 2019;37(19):2569–79. Available from: doi:https://doi.org/10.1016/j.vaccine.2019.03.070.
[32] Coler C, King-Nakaoka E, Every E, Chima S, Vong A, Del Rosario B, et al. Impact of infections during pregnancy on transplacental antibody transfer. Vaccines 2024;12(10):1–17.
[33] Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am J Reprod Immunol 1996;36(5):248–55.

[34] Ciobanu AM, Dumitru AE, Gica N, Botezatu R, Peltecu G, Panaitescu AM. Benefits and risks of IgG transplacental transfer. Diagnostics (Basel) [Internet] 2020;10 (583):1–21. Available from: www.mdpi.com/journal/diagnostics.

[35] Abu Raya B, Srugo I, Kessel A, Peterman M, Bader D, Gonen R, et al. The effect of timing of maternal tetanus, diphtheria, and acellular pertussis (Tdap) immunization during pregnancy on newborn pertussis antibody levels - a prospective study. Vaccine [Internet]. 2014;32(44):5787–93. Available from: doi: https://doi.org/10.1016/j.vaccine.2014.08.038.

[36] Eberhardt CS, Blanchard-Rohner G, Lemaître B, Combescure C, Othenin-Girard V, Chilin A, et al. Pertussis antibody transfer to preterm neonates after second-versus third-trimester maternal immunization. Clin Infect Dis [Internet] 2017;64(8): 1129–32. Available from: https://academic.oup.com/cid/article/64/8/1129/3069383.

[37] Vilajeliu A, Ferrer L, Munrós J, Goncé A, López M, Costa J, et al. Pertussis vaccination during pregnancy: antibody persistence in infants. Vaccine [Internet]. 2016;34(33):3719–22. Available from: doi:https://doi.org/10.1016/j.vaccine.2016.05.051.

[38] Atyeo CG, Shook LL, Brigida S, De Guzman RM, Demidkin S, Muir C, et al. Maternal immune response and placental antibody transfer after COVID-19 vaccination across trimester and platforms. Nat Commun [Internet] 2022;13(3571). Available from: doi:https://doi.org/10.1038/s41467-022-31169-8.

[39] Sartoretti J, Fontannaz P, Martinez De Tejada B, Othenin-Girard V, Chilin A, Lemaître B, et al. Influence of timing of maternal pertussis immunization on the avidity of transferred antibodies in term and preterm neonates. Clin Infect Dis 2023;77(4):645–8.