Pertussis immunization during pregnancy : assessment of the role of maternal antibodies on immune responses in term and preterm-born infants

Pertussis immunization during pregnancy: assessment of the role of maternal antibodies on immune responses in term and preterm born infants

Kirsten Maertens[1], Marjolein R.P. Orije[1], Sereina A. Herzog[2,3], Ludo M. Mahieu[4], Niel Hens[2,5], Pierre Van Damme[1], Elke Leuridan[1]

[1] Centre for the Evaluation of Vaccination, Vaccine & Infectious Diseases Institute, University of Antwerp, Antwerp, Belgium

[2] Centre for Health Economic Research and Modeling Infectious Diseases, Vaccine & Infectious Diseases Institute, University of Antwerp, Belgium

[3] Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Austria

[4] Department of Pediatrics, Division Neonatology, University Hospital Antwerp, University of Antwerp, Antwerp, Belgium

[5] Interuniversity Institute for Biostatistics and statistical Bioinformatics (I-BIOSTAT), Data Science Institute, Hasselt University, Hasselt, Belgium

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Running title: Maternal Tdap vaccination in preterms

Corresponding author:

Kirsten Maertens, PhD, Prof

Centre for the Evaluation of Vaccination, Vaccine & Infectious Diseases Institute, University of Antwerp, Belgium

Mail: kirsten.maertens@uantwerp.be

Alternate corresponding author:

Elke Leuridan, PhD, Prof

Centre for the Evaluation of Vaccination, Vaccine & Infectious Diseases Institute, University of Antwerp, Belgium

elke.leuridan@uantwerp.be

Summary:

- Preterm infants profit from maternal Tdap vaccination.
- Vaccination earlier in pregnancy is better to protect both term and preterm infants.
- Preterm infants mount favorable antibody-mediated immune responses in the presence of vaccine-induced maternal antibodies.

Abstract

Background: Limited data exist on the impact of maternal Tetanus, Diphtheria, acellular Pertussis (aP) (Tdap) vaccination for preterm born infants. We report its effect at birth and on antibody-mediated immune responses to a DTaP-IPV-HB-PRP~T vaccine in preterm compared to term infants.

Methods: Women delivering at term or prematurely were either vaccinated with a Tdap vaccine (Boostrix®, GSK) during pregnancy or not vaccinated in the last 5 years. Cord and maternal blood were collected at delivery. Infants were vaccinated with DTaP-IPV-HB-PRP~T vaccine (Hexyon®, Sanofi Pasteur) and bled before and one month after primary (8-12-16 weeks) and before and one month after booster vaccination (13 or 15 months for preterm and term respectively). Immunoglobulin G antibodies against all antigens included in DTaP-IPV-HB-PRP~T vaccine were measured (NCT02511327).

Results: Cord blood Geometric Mean Concentrations (GMCs) in preterm infants from Tdap-vaccinated women were significantly higher than in term and preterm infants from unvaccinated women. A longer time interval between maternal vaccination and delivery resulted in higher cord blood GMCs in preterm infants. Equal GMCs in term and preterm infants from Tdap-vaccinated women were observed after primary vaccination. After boosting, significantly lower GMCs were seen for PT, FHA and TT in preterm compared to term infants from Tdap-vaccinated women, yet still comparable to GMCs in both term and preterm infants from unvaccinated women.

Conclusion: Preterm infants profit from maternal Tdap vaccination. Prematurity did not influence primary immune responses in the presence of maternal antibodies, but was associated with a lower booster immune response.

Introduction

Despite the availability of universal pertussis vaccination programs [1], the disease remains an important public health problem [2]. Globally, in 2014, an estimated 24.1 million pertussis cases and 160,700 pertussis related deaths occurred in children younger than 5 years old with the highest incidence and mortality in the first year of life [3]. Additionally, preterm infants are more vulnerable for pertussis-related complications and are overrepresented among severe cases requiring hospitalization [4-6].

Maternal antibodies are transferred from the maternal to the fetal circulatory system across the placenta. These maternal antibodies offer protection to the infant against infectious diseases in the immediate postpartum period. The transplacental transport of maternal Immunoglobulin G (IgG) antibodies is regulated by the neonatal Fc receptor. Transport of antibodies increases with advancing gestation resulting in less efficient transport of IgG antibodies to preterm infants [7]. Maternal Tetanus, Diphtheria, acellular Pertussis (aP) (Tdap) vaccination enhances Tdap-specific antibody levels in term infants [8]. However, whether maternal Tdap vaccination could have a beneficial effect on the transfer of maternal antibodies to preterm infants is unknown.

High levels of maternal antibodies in newborns are associated with a reduced infant immune response, a phenomenon called blunting [9, 10]. This blunting has been extensively described for antibody responses to aP containing vaccines in term infants. However, limited data is available on this effect for preterm infants [11].

This study aims to answer the lack of knowledge on transplacental transport of vaccine-induced antibodies to preterm infants and on the potential difference in antibody-mediated immune responses between term and preterm infants to hexavalent aP containing infant vaccines in the presence of vaccine-induced maternal antibodies.

Material & Methods

Study design

A prospective observational study (NCT02511327) was conducted in Belgium.

Pregnant women and their offspring were divided in 4 different cohorts according to their gestational age (GA) at delivery and their vaccination status:

- VT cohort: women vaccinated with an aP containing vaccine during pregnancy and their term infants (≥37 weeks GA)
- VP cohort: women vaccinated with an aP containing vaccine during pregnancy and their preterm infants (<37 weeks GA)
- UnVT cohort: women not vaccinated with an aP containing vaccine for at least 5 years and their term infants
- UnVP cohort: women not vaccinated with an aP containing vaccine for at least 5 years and their preterm infants.

Mother-infant pairs were recruited through hospitals in Flanders, Belgium. GA was determined within routine prenatal care via first trimester ultrasound. Randomization was not possible since maternal Tdap vaccination is recommended in Belgium between 24-32 weeks GA. A list of inclusion/exclusion criteria is available in Appendix 1.

The study was approved by the University Hospital Antwerp ethics committee. Written informed consent was obtained from all participants and from both parents of the participating infants.

Study vaccines

Women in the VT and VP cohort received licensed Tdap vaccine (Boostrix®; GSK) during pregnancy. Boostrix® contains 5 limit of flocculation (Lf) Tetanus Toxoid (TT), 2.5Lf Diphtheria Toxoid (DT), 8µg inactivated Pertussis Toxin (PT), 8µg Filamentous Haemagglutinin (FHA) and 2.5µg of Pertactin (PRN).

All infants were vaccinated with a DTaP-IPV-HB-PRP~T vaccine (Hexyon®; Sanofi Pasteur) at 8-12-16 weeks (primary vaccination) and at 13 (preterm) or 15 (term) months of age (booster vaccination). Hexyon® contains not less than 40 international units (IU) TT, not less than 20IU DT, 25µg PT, 25µg FHA, 40D-antigen units poliovirus type 1, 8D-antigen units poliovirus type 2, 32D-antigen units poliovirus type 3, 10µg Hepatitis B surface antigen and 12µg *Hemophilus influenzae* type b polysaccharide conjugated to 22-36µg tetanus protein.

According to the Belgian national immunization schedule, infants also received the 10-valent pneumococcal polysaccharide vaccine (Synflorix®; GlaxoSmithKline Biologicals) at 8 and 16 weeks (with an extra dose for preterms at 12 weeks) and 12 months; the measles-mumps-rubella vaccine (MMRVaxPro®; Merck) at 12 months, the Meningococcal C vaccine (Neisvac-C®; Pfizer) at 13 (preterm) or 15 (term) months and the optional rotavirus vaccine (Rotarix®; GSK or Rotateq®; Merck) at 8-(12)-16 weeks.

Study procedures

All vaccines in the study were administered within the regular healthcare system. Maternal demographic data were collected at baseline. At each follow-up visit, a questionnaire on demographics, diseases and drug use, growth parameters, breastfeeding status, immunization data, day-care attendance and respiratory infection episodes in the household was collected.

Blood samples were collected from all participating women and infants (cord) at birth. Infant blood samples were collected before (8 weeks ± 4 days) and one month (28-35 days) after primary vaccination and before (1-14 days) and one month (28-35 days) after booster vaccination.

Blood samples were centrifuged for 10 minutes at 1300g within 24 hours after sample collection and stored at -35°C.

Laboratory

All samples were tested at the Global Clinical Immunology laboratory of Sanofi Pasteur in Swiftwater, Pennsylvania. Sera were tested for IgG antibodies against PT, FHA, PRN, TT and DT by an in-house electrochemiluminescent method using a Meso Scale Discovery (MSD) technology. Antibodies against Hepatitis B (Hep B) were evaluated by the commercially available VITROS ECi HBs assay and levels of anti-*Haemophilus influenzae* type b (Hib) antibodies were determined by Radioimmunoassay. Levels against poliovirus were evaluated with a Micrometabolic Inhibition test but are not presented due to the low number of samples tested.

Samples with values below the assays lower limit of quantification (LLOQ) were assigned 50% of the LLOQ. The respective LLOQ were 2EU/mL for PT, FHA and PRN; 0.01IU/mL for TT; 0.005IU/mL for DT; 5mIU/mL for Hep B and 0.06µg/mL for Hib.

Statistics

A sample size calculation was performed based on a previous study [12], taking into account a power of 90%, a fixed variance and 10% loss to follow-up. Baseline characteristics were reported as mean and standard deviation or as absolute numbers and percentages. Comparison of baseline characteristics was done using either T-, Mann-Whitney-U or Fisher-Exact test. Antigen specific GMCs and 95% confidence intervals (CI) were calculated at each timepoint in all study groups. Blunting of infant immune responses was defined as a statistically significantly lower GMC after primary or booster vaccination in infants from Tdap vaccinated women compared to infants from unvaccinated women. Transplacental transport ratio was calculated as the ratio of the raw cord blood levels divided by the raw maternal antibody levels at delivery.

We estimated the half-life of antibodies using the measurements at birth and before primary vaccination fitting linear mixed-effect (LME) models of log2-transformed antibody concentrations. The antibody half-life is given as the inverse of the regression line slope expressed in days. We investigated differences between the cohorts for the predefined outcomes (e.g. transplacental transport ratio) and potential determinants (e.g. GA at delivery) using generalized estimating equations (GEE) models checking for determinant interactions. An exchangeable correlation structure was used to take account pairs of twins. GEE models were compared using the quasi information criteria (QIC) [12]. All mentions of (non)-significant results are based on the GEE models except comparison in demographic data and half-life estimates.

Statistical analyses were performed using the statistical software R (version 4.0.2) with package geepack (version-1.3-1) for the GEE and lme4 (version-1.1-23) for the LME models. Two-sided p-value <0.05 was considered statistically significant.

Results

Demographics

We discuss all available data, since no differences between all available data and data with full protocol adherence were present. Overall, 234 pregnant women were enrolled between May 19th, 2015 and June 26th, 2017. From these women, 231 infants were included and assigned to the different cohorts (Supplementary Figure 1). Characteristics of mother-infant pairs are shown in Table 1. Substantial differences in characteristics existed only for those related to preterm delivery (Table 1).

Transplacental transport

No significant difference in transplacental transport ratio was observed between vaccinated and unvaccinated cohorts (VT vs UnVT/VP vs UnVP) whereas a significantly lower transplacental transport ratio was observed in preterm versus term cohorts after maternal immunization (VT vs VP) for all Tdap-included antigens (Table 2).

An association between GA at delivery and transplacental transport ratio was noted for all Tdap-included antigens with a trend towards a transplacental transport ratio >1 after 34 weeks GA (Supplementary Figure 2).

A significant association between GA at vaccination and transplacental transport ratio was observed for both term and preterm deliveries with a lower ratio if the GA at vaccination increases. Additionally, a longer interval between vaccination and delivery was significantly correlated with a higher transplacental transport ratio (Supplementary Figure 3).

Impact of timing of maternal vaccination

No association between GA at vaccination and cord blood GMCs was found for both term and preterm infants for PT, FHA and TT. For PRN and DT, an association between GA at vaccination and cord blood GMCs was present only in the preterm cohorts with lower levels if the GA at vaccination increased (Figure 2A & Supplementary Figure 4).

For all Tdap-included antigens (except TT), interval between vaccination and delivery significantly correlates with cord blood GMCs in preterm but not term infants with significantly higher levels if the interval between vaccination and delivery increased (Figure 2B & Supplementary Figure 4).

Immunogenicity Tdap-included antigens

Term infants from vaccinated women (VT) had significantly higher cord blood GMCs at birth compared to preterm infants from vaccinated women (VP) for all Tdap-included antigens. These significantly higher GMCs remained until the start of primary vaccination, except for TT. No significant difference in GMCs was observed between term and preterm infants from vaccinated women after primary vaccination for all antigens, nor before the booster. After booster vaccination, term infants of vaccinated women had significantly higher GMCs for PT, FHA and TT compared to preterm infants from vaccinated women. For DT, comparable GMCs were seen after boosting (Table 3 & Figure 3).

Significantly higher half-life estimates for Tdap-specific antibodies induced by maternal immunization were observed in preterm versus term infants (Supplementary Table 2).

When comparing term (UnVT) and preterm infants (UnVP) from unvaccinated women, comparable GMCs were seen at birth for all Tdap-included antigens. Before and after primary vaccination, significantly higher GMCs for TT were seen in term versus preterm infants from

unvaccinated women. For all other antigens, comparable GMCs were observed before and after primary and booster vaccination (Table 3 & Figure 3).

Term infants from vaccinated (VT) women have significantly higher GMCs in cord blood and before primary vaccination compared to term infants from unvaccinated women (UnVT) for all Tdap-included antigens. After primary vaccination, blunting was observed for FHA and DT. For PT, PRN and TT, comparable GMCs were seen after priming. Before booster vaccination, comparable GMCs were measured between term infants from vaccinated and unvaccinated women for PT, DT and FHA. For TT, significantly higher GMCs were present in term infants from vaccinated women. After boosting, significantly higher GMCs were observed in term infants from vaccinated women for PT and TT. In contrast, for DT, significantly lower GMCs were seen in term infants from vaccinated versus unvaccinated women suggesting persistence of blunting. For FHA, no differences were observed after boosting (Table 3 & Figure 3).

When considering preterm infants from vaccinated (VP) and unvaccinated women (UnVP), significantly higher GMCs were present in cord blood and before primary vaccination in preterm infants from vaccinated women for all Tdap-included antigens, except for TT in cord blood. Blunting was observed for DT after primary vaccination. For all other antigens, no blunting was present in preterm infants. Before and after booster vaccination, comparable GMCs were measured for all Tdap-included antigens (Table 3 & Figure 3).

Association between pre-existing antibody levels and infant primary immune responses

In both term (VT) and preterm (VP) infants from vaccinated women, an association between cord blood and post-primary vaccination GMCs was observed for PT and DT with lower post-primary GMCs if cord blood antibody GMCs were higher. For FHA, this effect was only present in preterm infants.

Associating pre- and post-primary GMCs in infants from vaccinated women, significantly lower post-primary GMCs were found for PT in term and preterm infants if pre-primary GMCs were higher. This observation was also present for DT, but only in preterms.

Immunogenicity other antigens

Comparable GMCs between term (VT) and preterm (VP) infants from vaccinated women were observed for Hib before primary vaccination. After primary vaccination, significantly higher GMCs for Hib were seen in term versus preterm infants from vaccinated women, persisting before and after booster vaccination. For Hep B, comparable GMCs between term and preterm infants from vaccinated women were present at all time points.

When comparing term (UnVT) and preterm (UnVP) infants from unvaccinated women, a significantly higher GMC was only observed in term versus preterm infants for Hep B before booster vaccination. For Hib, no differences were observed between term and preterm infants from unvaccinated women at all time points.

No differences in GMCs between term infants from vaccinated (VT) and unvaccinated (UnVT) women were observed for both Hib and Hep B at all time points. When considering preterm infants from vaccinated (VP) and unvaccinated (UnVP) women, only a significant higher GMC was observed for Hep B in infants from vaccinated women after booster vaccination (Table 3 & Figure 3)

Discussion

Limited data on the impact of maternal Tdap vaccination in preterm infants, who are at higher risk for pertussis-related complications [13], are available. Our study investigated the effect of maternal Tdap vaccination on antibody levels against six antigens included in the DTaP-IPV-HB-PRP~T infant vaccine in cord blood and before and after primary and booster vaccination in term and preterm infants.

The study showed that preterm infants profit from maternal Tdap vaccination and that vaccination earlier in pregnancy is more advantageous. Despite the reduced transplacental transport linked to preterm delivery, significantly higher antibody levels at birth and before primary vaccination are observed in preterm infants from vaccinated women compared to both term and preterm infants from unvaccinated women for all Tdap-included antigens.

Compromised transplacental transport associated with preterm delivery has already been described [7, 14]. However, our study is the first to confirm this observation in the presence of maternal Tdap vaccination. In accordance with previous research [9, 15, 16], no influence of maternal immunization status on transplacental transport was found for both term and preterm deliveries.

In determining the effect of timing of maternal vaccination on infant antibody levels at birth, GA at vaccination is usually used [17-20]. However, in preterm infants, the interval between vaccination and delivery seems more adequate. We report that a longer interval between vaccination and delivery was significantly associated with a higher transplacental transport ratio in both term and preterm cohorts and with higher cord blood antibody levels in preterm infants. This confirms data from previous studies [17-19, 21] that vaccination earlier in pregnancy is more beneficial to achieve high antibody levels at birth. Recently, a UK study showed that extending the vaccination window results in a decrease of the number of hospitalized pertussis cases in preterm infants by half [22]. Therefore, we can conclude that

expediting the window of vaccination to the second trimester of pregnancy is preferred to protect both term and preterm infants during early life.

In our study, blunting was observed after primary vaccination for FHA and DT in term infants born to vaccinated versus unvaccinated women, as previously described [9, 15, 16, 23]. After booster vaccination, persistence of blunting in term infants was observed for DT. However, all infants achieved seroprotective levels for DT after boosting, illustrating no increase in susceptibility due to this blunting.

When considering preterm infants born to vaccinated versus unvaccinated women, blunting was only observed for DT after primary vaccination and no blunting was present after boosting. However, looking at the association between pre-existing antibody levels and post-primary GMCs in preterm infants from vaccinated women, a significant association was found for PT, FHA and DT, indicating that preterm infants starting primary vaccination with high antibody levels are more likely to experience a modulation of their immune response.

Contradictory, Kent et al. [11] found blunting in preterm infants after primary vaccination for FHA and DT. The absence of blunting for FHA in preterm infants in our study could be due to the smaller sample size of our unvaccinated preterm cohort or due to the use of different vaccine brands in women and infants. Important side note is that the study by Kent et al. was originally not designed for evaluation of maternal Tdap vaccination. Additionally, no evaluation of booster responses in the second year of life was performed. Similar to the studies by Barug et al and Rice at al [24, 25], no impact of maternal Tdap vaccination on antibody responses to Hib has been observed in this study which is in contradiction to Ladhani et al describing an enhancement of the Hib primary immune response after maternal Tdap vaccination [26].

Important to highlight is that the present study reports the first data on the use of the DTaP-IPV-HB-PRP~T vaccine (Hexyon®) in preterm infants. We report comparable GMCs in term and preterm infants after a 3-dose primary series. After boosting however, term infants from Tdap-vaccinated women have significantly higher GMCs for PT, FHA and TT compared to their preterm counterparts. Since preterm infants received their booster vaccination at an earlier age compared to term infants, the question is whether the difference in booster response in infants from Tdap-vaccinated women is caused by the presence of more circulating maternal antibodies or the immunological immaturity when vaccinating at an earlier age. However, GMCs in preterm infants from vaccinated women were comparable with GMCs in both term and preterm infants from unvaccinated women supporting the immunogenicity of the Hexyon® vaccine for both primary and booster vaccination in preterm infants.

This large prospective cohort study reports on the impact of maternal Tdap vaccination on preterm infants. Yet, this study has some limitations including the small sample size of the unvaccinated cohorts, probably due to the high compliance of women to maternal Tdap vaccination with a coverage of ±70% in Flanders [27, 28]. As such, we were not able to reach our target sample size of the unvaccinated cohorts making it difficult to draw firm conclusions on comparisons with these cohorts. Another limitation is the different timing of the booster dose in term versus preterm infants. However, preterm infants are vaccinated at an earlier calendar age within the national immunization schedule to offer them better protection against infectious disease, postponing their vaccination within the study would not be ethical and thus not possible.

In conclusion, we showed within this study that preterm infants profit from maternal Tdap vaccination and that vaccination earlier in pregnancy is preferred to protect both term and preterm born infants.

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Conflict of interest

None of the authors have a conflict of interest to declare for this manuscript.

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		VT Cohort	VP Cohort	UnVT Cohort	UnVP Cohort
Women	**N (women)**	112	82	22	18
	Mean age at delivery in years (SD)	31.4 (3.7)	30.9 (4.3)	30.5 (3.8)	31.8 (6.2)
	Race, N (%)				
	Caucasian	106 (94.6)	79 (96.3)	20 (90.9)	16 (88.9)
	Non-Caucasian	6 (5.4)	3 (3.7)	2 (9.1)	2 (11.1)
	Educational level, N (%)				
	Primary school	0 (0.0)	0 (0.0)	1 (4.5)	0 (0.0)
	Secondary School	13 (11.6)	12 (14.7)	4 (18.2)	8 (44.4)
	Bachelor	48 (42.9)	36 (43.9)	6 (27.3)	3 (16.7)
	Master	40 (35.7)	27 (32.9)	7 (31.8)	3 (16.7)
	PhD	4 (3.6)	1 (1.2)	0 (0.0)	0 (0.0)
	Unknown	7 (6.2)	6 (7.3)	4 (18.2)	4 (22.2)
	Mean GA at delivery in weeks (Min-Max, SD)	39.6 (37.0-41.6; 1.2)	34.0 (28.4-36.9; 2.3)	39.1 (37.0-40.7; 1.0)	31.8 (24.1-36.7; 3.9)
	Mean GA at vaccination in weeks (Min-Max, SD)	29.3 (13.4-36.9; 3.7)*	28.8 (23.4-35.9; 2.5)	NA	NA
	Trimester of vaccination, N (%)			NA	NA
	Unknown	3* (2.7)	0 (0.0)		
	First trimester (<14 weeks GA)	1 (0.9)	0 (0.0)		
	Second trimester (≥14 -<27 weeks GA)	22 (19.6)	21 (25.6)		
	Third trimester (≥27 weeks GA)	86 (76.8)	61 (74.4)		
	Mean interval between maternal vaccination and delivery in weeks (Min-Max; SD)	10.3 (1.6-26.1; 3.9)	5.4 (0.4-13.0; 2.9)	NA	NA
	Parity, N (%)				
	Uniparous	64 (57.1)	59 (72.0)	10 (45.5)	10 (55.6)
	Multiparous	48 (42.9)	23 (28.0)	12 (54.5)	8 (44.4)
	Induction of labor, N (%)				
	Yes	36 (32.1)	16 (19.5)	7 (31.8)	4 (22.2)
	No	75 (67.0)	66 (80.5)	15 (68.2)	13 (72.2)
	Unknown	1 (0.9)	0 (0.00)	0 (0.00)	1 (5.6)
	Epidural Anesthesia, N (%)				
	Yes	72 (64.3)	57 (69.5)	14 (63.6)	8 (44.4)
	No	37 (33.0)	21 (25.6)	8 (36.4)	9 (50.0)
	Unknown	3 (2.7)	4 (4.9)	0 (0.00)	1 (5.6)
	Mode of delivery, N (%)				
	Vaginal	85 (75.9)	44 (53.7)	15 (68.2)	12 (66.7)
	C-section	26 (23.2)	38 (46.3)	7 (31.8)	6 (33.3)
	Unknown	1 (0.9)	0 (0.00)	0 (0.00)	0 (0.0)
Infants	**N (included infants)**	**109 (2 twins)**	**89 (18 twins)**	**18**	**15 (1 twin)**

	Ethnicity, N (%) Caucasian Non-Caucasian	 99 (90.8) 10 (9.2)	 83 (93.3) 6 (6.7)	 15 (83.3) 3 (16.7)	 14 (93.3) 1 (6.7)
	Sex, N (%) Male Female	 51 (46.8) 58 (53.2)	 47 (52.8) 42 (47.2)	 10 (55.6) 8 (44.4)	 11 (73.3) 4 (26.7)
	Breastfeeding at birth, N (%) Yes No	 97 (89.0) 12 (11.0)	 79 (88.8) 10 (11.2)	 13 (72.2) 5 (27.8)	 14 (93.3) 1 (6.7)
	Mean duration of breastfeeding in weeks (SD)	25.3 (22.5)	16.0 (13.7)	32.3 (23.9)	17.5 (15.7)
	Stayed in the neonatal care unit, N (%) Yes No Unknown	 6 (5.5) 101 (92.7) 2 (1.8)	 76 (85.4) 13 (14.6) 0 (0.00)	 1 (5.6) 15 (83.3) 2 (11.1)	 12 (80.0) 3 (20.0) 0 (0.0)
	Mean duration of stay at neonatal care unit in days (SD)	4.5 (3.8)	26.5 (19.1)	19.0 (NA)	41.3 (19.6)
	Mean birth weight in grams (SD)	3439.4 (442.7)	2212.6 (572.3)	3355.3 (519.5)	1815.0 (616.3)
	Mean weight before primary vaccination in grams (SD)	5139.8 (648.9)	3871.1 (801.1)	4891.3 (893.2)	3511.4 (980.8)
	Mean weight one month after primary vaccination in grams (SD)	7399.8 (902.3)	6493.3 (1003.4)	7090.8 (1075.9)	6095.5 (1062.7)
	Mean weight before booster vaccination in grams (SD)	10355.9 (1071.7)	9387.2 (1208.2)	10164.2 (829.9)	8855.5 (1300.7)
	Mean weight one month after booster vaccination in grams (SD)	10456.7 (1050.3)	9641.0 (1275.1)	10149.2 (836.6)	9150.5 (1346.2)
	Mean age at blood sampling before primary vaccination in weeks (SD)	8.0 (0.3)	7.9 (0.3)	7.9 (0.4)	8.0 (0.5)
	Mean age at blood sampling one month after primary vaccination in weeks (SD)	23.3 (2.2)	23.0 (1.8)	24.1 (3.0)	23.5 (2.1)
	Mean age at blood sampling before booster vaccination in months (SD)	15.0 (0.4)	13.5 (0.7)	15.00 (0.5)	13.4 (0.6)
	Mean age at blood sampling one month after booster vaccination in months (SD)	16.4 (0.5)	14.9 (0.9)	16.2 (0.6)	14.7 (0.7)
	Mean age at hexavalent vaccine dose 1 in weeks (SD)	9.0 (0.9)	9.0 (0.9)	9.4 (1.3)	9.1 (1.0)
	Mean age at hexavalent vaccine dose 2 in weeks (SD)	13.7 (1.3)	13.8 (1.3)	14.1 (1.7)	14.0 (1.1)
	Mean age at hexavalent vaccine dose 3 in weeks (SD)	18.9 (2.2)	18.7 (1.9)	19.3 (2.7)	19.4 (2.0)
	Mean interval between primary vaccination and blood sampling in days (SD)	29.7 (5.9)	30.9 (2.8)	34.8 (11.1)	31.4 (4.3)
	Mean age at hexavalent vaccine dose 4 in months (SD)	15.3 (0.5)	13.9 (0.9)	15.2 (0.6)	13.7 (0.8)
	Mean interval between booster vaccination and blood sampling in days (SD)	31.7 (4.8)	31.9 (5.0)	31.3 (3.1)	29.2 (2.8)

Table 1: Demographic characteristics study participants. * 3 women with unknown vaccination date

Antigen	Mean Fetal-to-Maternal Antibody Ratio at Delivery (95% CI)			
	VT Cohort N=95	VP Cohort N=38 (37 for PT, PRN, TT)	UnVT Cohort N=15	UnVP Cohort N=5
Pertussis Toxoid	1.60 (1.44-1.76)	0.92 (0.75-1.09)	1.95 (1.51-2.39)	1.22 (0.97-1.47)
Filamentous Hemagglutinin	1.69 (1.58-1.81)	0.97 (0.80-1.14)	1.62 (1.32-1.93)	1.18 (0.93-1.42)
Pertactin	1.84 (1.21-2.47)	0.91 (0.72-1.09)	1.58 (1.29-1.87)	1.20 (0.96-1.44)
Tetanus Toxoid	1.63 (1.53-1.74)	1.05 (0.86-1.23)	1.57 (1.27-1.86)	1.18 (0.95-1.41)
Diphtheria Toxoid	1.53 (1.42-1.63)	0.96 (0.77-1.14)	1.47 (1.24-1.69)	1.04 (0.82-1.26)

Table 2: Mean Fetal-to-Maternal Antibody Ratio at Delivery with 95% confidence interval

GMC (95%CI)	Women at delivery	Cord	Before primary vaccination	1 month after primary vaccination	Before booster vaccination	1 month after booster vaccination
VT cohort (N)	**106**	**100**	**102** **(74 for Hib; 72 for Hep B)**	**93** **(61 for Hib; 50 for Hep B)**	**94** **(93 for PT; 74 for Hib; 79 for Hep B)**	**99** **(71 for Hib; 52 for Hep B)**
Anti-PT	59.69 (50.24-70.92)	87.83 (73.07-105.57)	18.70 (15.23-22.95)	44.57 (39.30-50.54)	5.89 (4.93-7.04)	79.97 (68.07-93.95)
Anti-FHA	231.49 (195.47-274.16)	365.80 (307.11-435.70)	80.84 (66.30-98.56)	120.28 (107.37-134.73)	20.78 (17.64-24.47)	146.98 (125.28-172.43)
Anti-PRN	250.90 (199.65-315.31)	387.06 (307.00-488.02)	74.93 (57.81-97.12)	NA	NA	NA
Anti-TT	4.18 (3.63-4.82)	6.36 (5.43-7.44)	1.29 (1.09-1.53)	1.20 (1.05-1.38)	1.95 (1.61-2.38)	12.75 (11.02-14.75)
Anti-DT	0.90 (0.74-1.10)	1.24 (1.00-1.53)	0.28 (0.23-0.34)	0.21 (0.18-0.24)	0.20 (0.15-0.25)	1.93 (1.63-2.29)
Anti-Hib	NA	NA	0.12 (0.09-0.17)	1.57 (1.06-2.30)	0.44 (0.29-0.68)	39.07 (27.27-55.98)
Anti-Hep B	NA	NA	13.79 (8.89-21.38)	105.88 (74.54-150.40)	31.14 (21.82-44.44)	290.89 (174.08-486.08)
VP cohort (N)	**63**	**54** **(53 for PT and TT)**	**83** **(50 for Hib; 63 for Hep B)**	**69** **(35 for Hib; 46 for Hep B)**	**76** **(55 for Hib; 40 for Hep B)**	**76** **(51 for Hib; 35 for Hep B)**
Anti-PT	58.18 (44.93-75.32)	47.64 (37.93-59.84)	10.94 (8.55-14.00)	42.90 (36.33-50.64)	6.08 (5.02-7.36)	54.75 (45.47-65.92)
Anti-FHA	178.89 (133.89-239.02)	143.53 (110.99-185.61)	43.77 (33.35-57.45)	106.69 (90.60-125.65)	21.14 (17.39-25.71)	98.18 (81.53-118.24)
Anti-PRN	217.80 (140.53-337.55)	171.84 (113.91-259.24)	42.40 (29.80-60.31)	NA	NA	NA
Anti-TT	4.63 (3.67-5.84)	4.34 (3.70-5.09)	1.06 (0.86-1.31)	1.04 (0.88-1.22)	1.93 (1.55-2.41)	8.52 (7.19-10.10)
Anti-DT	0.76 (0.55-1.06)	0.67 (0.50-0.91)	0.17 (0.12-0.22)	0.28 (0.22-0.35)	0.25 (0.18-0.34)	1.55 (1.27-1.89)
Anti-Hib	NA	NA	0.09 (0.06-0.12)	0.70 (0.42-1.16)	0.21 (0.13-0.32)	11.94 (7.72-18.46)
Anti-Hep B	NA	NA	12.14 (7.58-19.45)	111.65 (73.27-170.12)	25.88 (15.48-43.26)	260.91 (161.64-421.14)

UnVT cohort (N)	**17**	**17**	**14** **(4 for Hib; 10 for Hep B)**	**13** **(5 for Hib; 10 for Hep B)**	**11** **(7 for Hib; 9 for Hep B;)**	**13** **(12 for DT; 7 for Hib; 3 for Hep B)**
Anti-PT	8.32 (4.50-15.37)	15.37 (8.83-26.75)	3.39 (1.97-5.82)	56.65 (44.51-72.09)	6.40 (3.62-11.33)	48.83 (37.15-64.20)
Anti-FHA	22.19 (13.50-36.50)	42.70 (29.76-61.26)	13.21 (9.42-18.52)	162.11 (128.97-203.77)	23.61 (14.68-37.98)	122.70 (95.91-156.97)
Anti-PRN	19.08 (7.78-46.77)	39.79 (19.89-79.58)	11.67 (4.91-27.74)	NA	NA	NA
Anti-TT	1.07 (0.76-1.51)	1.66 (1.20-2.30)	0.52 (0.42-0.64)	0.82 (0.56-1.20)	0.77 (0.43-1.37)	5.59 (3.18-9.84)
Anti-DT	0.06 (0.03-0.12)	0.11 (0.06-0.20)	0.04 (0.02-0.08)	0.40 (0.23-0.69)	0.15 (0.06-0.35)	1.44 (0.81-2.56)
Anti-Hib	NA	NA	0.09 (0.02-0.33)	1.64 (0.36-7.58)	0.14 (0.04-0.51)	10.98 (2.46-49.02)
Anti-Hep B	NA	NA	16.61 (5.84-47.24)	209.94 (108.22-407.27)	54.16 (19.50-150.43)	89.62 (9.80-819.74)
UnVP cohort (N)	**14**	**6**	**14 (9 for Hep B and Hib)**	**10** **(3 for Hib; 7 for Hep B)**	**10** **(7 for Hib; 8 for Hep B)**	**11** **(8 for Hib; 7 for Hep B;)**
Anti-PT	6.04 (2.95-12.37)	6.91 (2.26-21.13)	2.26 (1.38-3.71)	46.13 (24.95-85.28)	8.04 (4.59-14.06)	55.73 (28.04-110.78)
Anti-FHA	16.05 (6.85-37.59)	29.03 (11.63-72.44)	5.04 (2.52-10.05)	91.67 (59.69-140.78)	24.26 (15.20-38.74)	96.72 (52.24-179.08)
Anti-PRN	9.27 (4.49-19.12)	15.90 (7.06-35.81)	3.50 (1.95-6.27)	NA	NA	NA
Anti-TT	0.87 (0.42-1.82)	1.39 (0.33-5.90)	0.22 (0.11-0.43)	1.48 (0.85-2.56)	1.59 (0.83-3.05)	10.39 (6.58-16.40)
Anti-DT	0.09 (0.04-0.20)	0.09 (0.03-0.30)	0.01 (0.01-0.03)	0.61 (0.32-1.18)	0.29 (0.16-0.55)	2.49 (1.36-4.57)
Anti-Hib	NA	NA	0.05 (0.03-0.08)	1.20 (0.05-26.31)	0.41 (0.14-1.22)	12.10 (5.27-27.75)
Anti-Hep B	NA	NA	11.23 (3.00-42.00)	57.68 (15.78-210.77)	8.22 (2.46-27.47)	26.10 (5.75-118.50)

Table 3: Geometric Mean Concentration (GMC) with 95% confidence interval (CI) for antibodies against PT, FHA, PRN, TT, DT, Hep B and Hib in ELISA Units per milliliter (EU/mL) for PT, FHA and PRN, in International Units per milliliter (IU/mL) for TT and DT, in µg/mL for Hib and in mIU/mL for Hep B at all timepoints in all study groups.

Figure legends

Figure 1: Schematic overview of vaccinations and blood samplings for all cohorts (*for preterm born infants)

Figure 2: The impact of gestational age at vaccination in weeks (A) and interval between vaccination and delivery in weeks (B) on cord blood antibody levels (PT as an example; same trend observed for other Tdap-included antigens). Lines show population-averaged regression results from the GEE model. Lines are only presented in case of a significant effect.

Figure 3: Antibody concentrations in infants at all study time points. Anti-pertussis toxin (PT) (A), anti-filamentous haemagglutinin (FHA) (B), anti-pertactin (PRN) (C), anti-diphtheria (DT) (D), anti-tetanus (TT) (E), anti-Haemophilus Influenzae Type B (Hib) (F) and anti-Hepatitis B (Hep B) (G) antibody concentrations in term infants from vaccinated women (VT cohort), preterm infants from vaccinated women (VP cohort), term infants from unvaccinated women (UnVT cohort) and preterm infants from unvaccinated women (UnVP cohort) at all study time points. Results represent individual antibody concentrations on a natural logarithmic scale, expressed in EU/mL for PT, FHA and PRN, in IU/mL for DT and TT, in μg/mL for Hib and in mIU/mL for Hep B. Box plots are displayed: middle lines inside the rectangles represent median values and horizontal lines of rectangles represent lower and upper quartiles of distributions.

Supplementary Figure 1: Flowchart of the study

Supplementary Figure 2: The impact of gestational age at delivery on transplacental transport ratio (PT as an example). Lines show population-averaged regression results from the GEE model.

Supplementary Figure 3: The impact of gestational age at vaccination in weeks (A) and interval between vaccination and delivery in weeks (B) on transplacental transport ratio (PT as an example). Lines show population-averaged regression results from the GEE model.

Supplementary Figure 4: The impact of gestational age at vaccination in weeks (A/C/E/G/I) and interval between vaccination and delivery in weeks (B/D/F/H/J) on cord blood antibody levels for all Tdap-included antigens. Lines show population-averaged regression results from the GEE model. Lines are only presented in case of a significant effect. Panel A and B are also presented in Figure 2 of the main manuscript.

Antigen	Mean Fetal-to-Maternal Antibody Ratio at Delivery (95% CI)			
	VT Cohort N=95	VP Cohort N=38 (37 for PT, PRN, TT)	UnVT Cohort N=15	UnVP Cohort N=5
Pertussis Toxin~~oid~~	1.60 (1.44-1.76)	0.92 (0.75-1.09)	1.95 (1.51-2.39)	1.22 (0.97-1.47)
Filamentous Hemagglutinin	1.69 (1.58-1.81)	0.97 (0.80-1.14)	1.62 (1.32-1.93)	1.18 (0.93-1.42)
Pertactin	1.84 (1.21-2.47)	0.91 (0.72-1.09)	1.58 (1.29-1.87)	1.20 (0.96-1.44)
Tetanus Toxoid	1.63 (1.53-1.74)	1.05 (0.86-1.23)	1.57 (1.27-1.86)	1.18 (0.95-1.41)
Diphtheria Toxoid	1.53 (1.42-1.63)	0.96 (0.77-1.14)	1.47 (1.24-1.69)	1.04 (0.82-1.26)

Table 2: Mean Fetal-to-Maternal Antibody Ratio at Delivery with 95% confidence interval

Figure 2

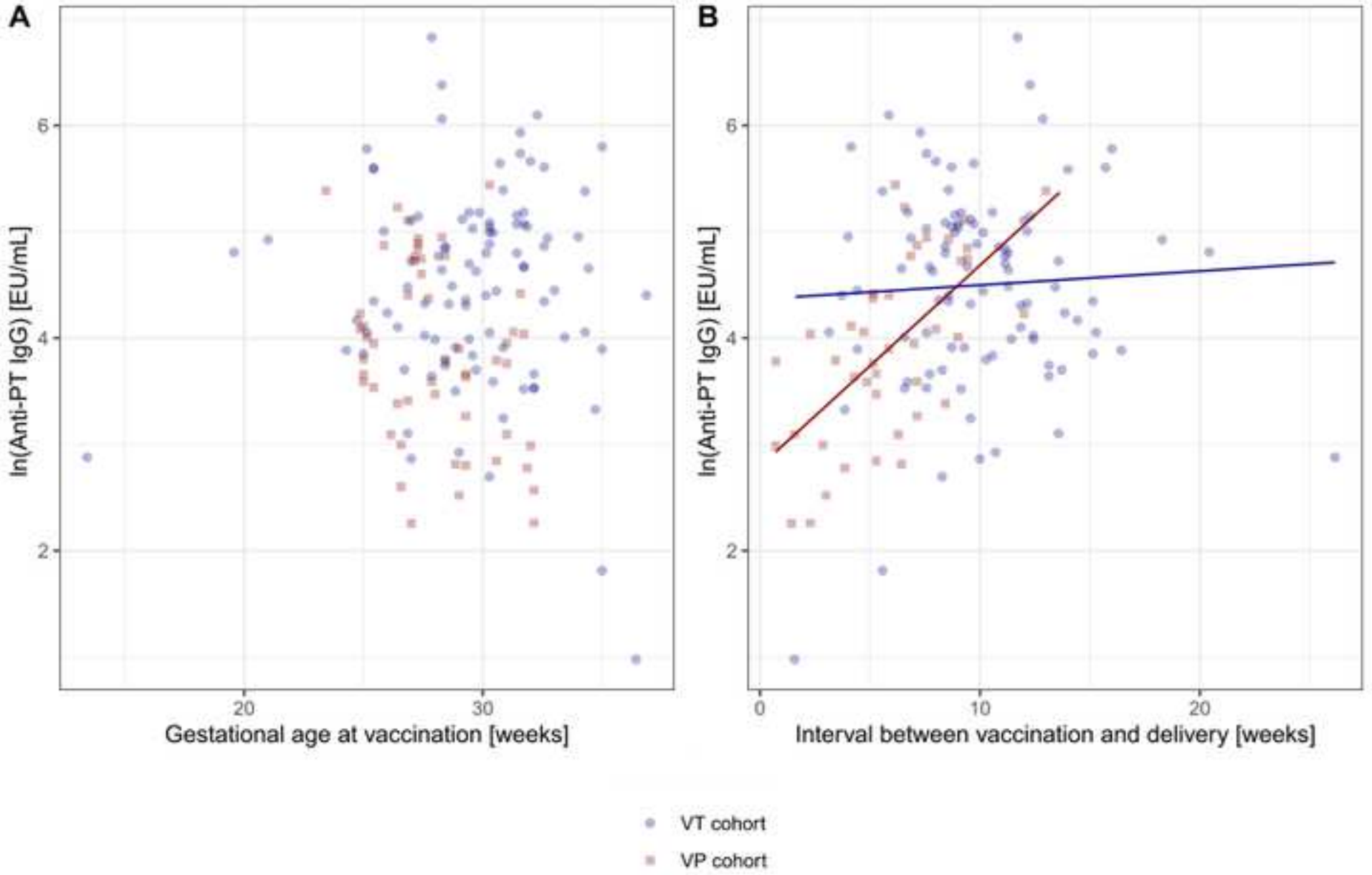

Figure 3

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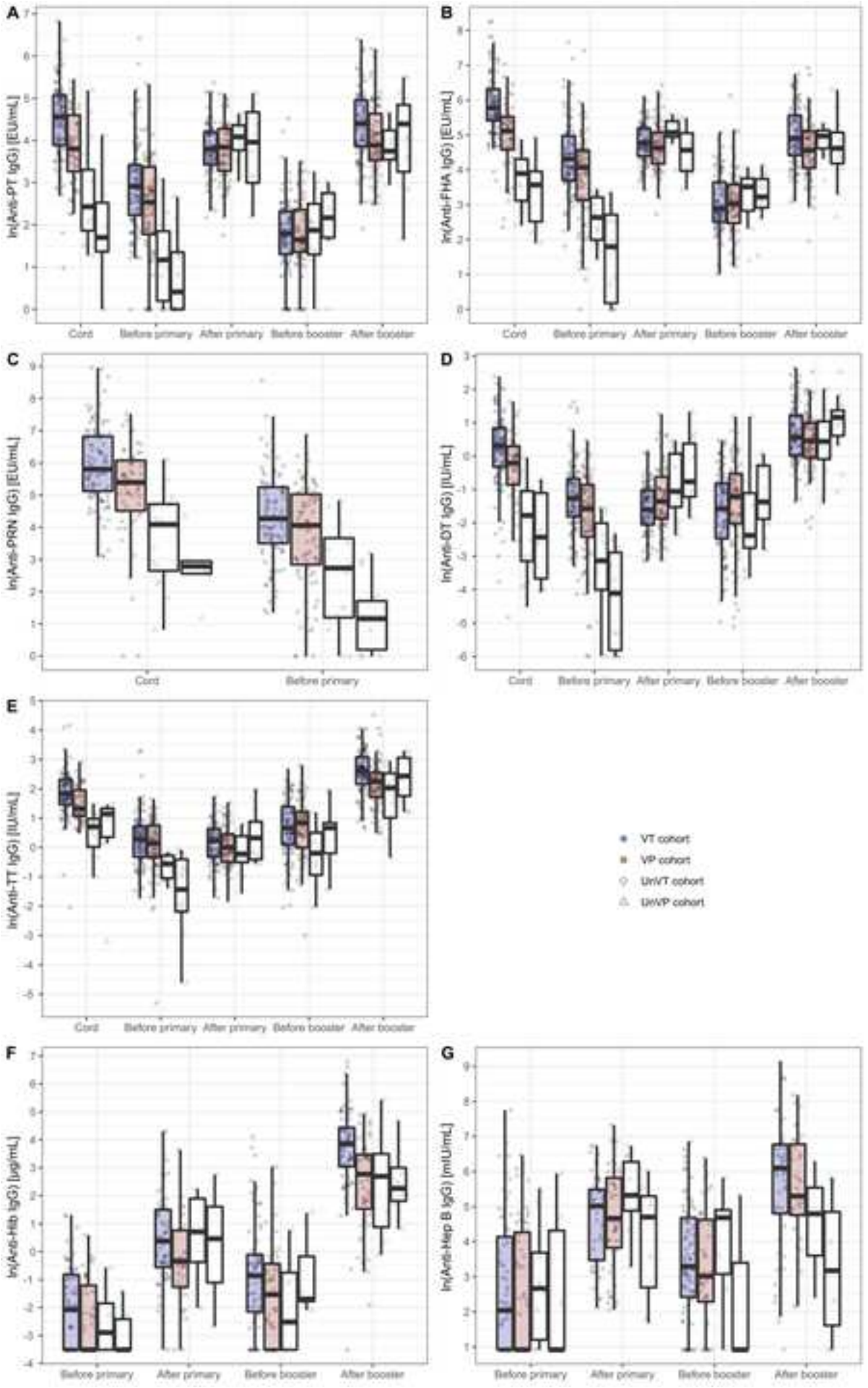

Supplementary Figure 1

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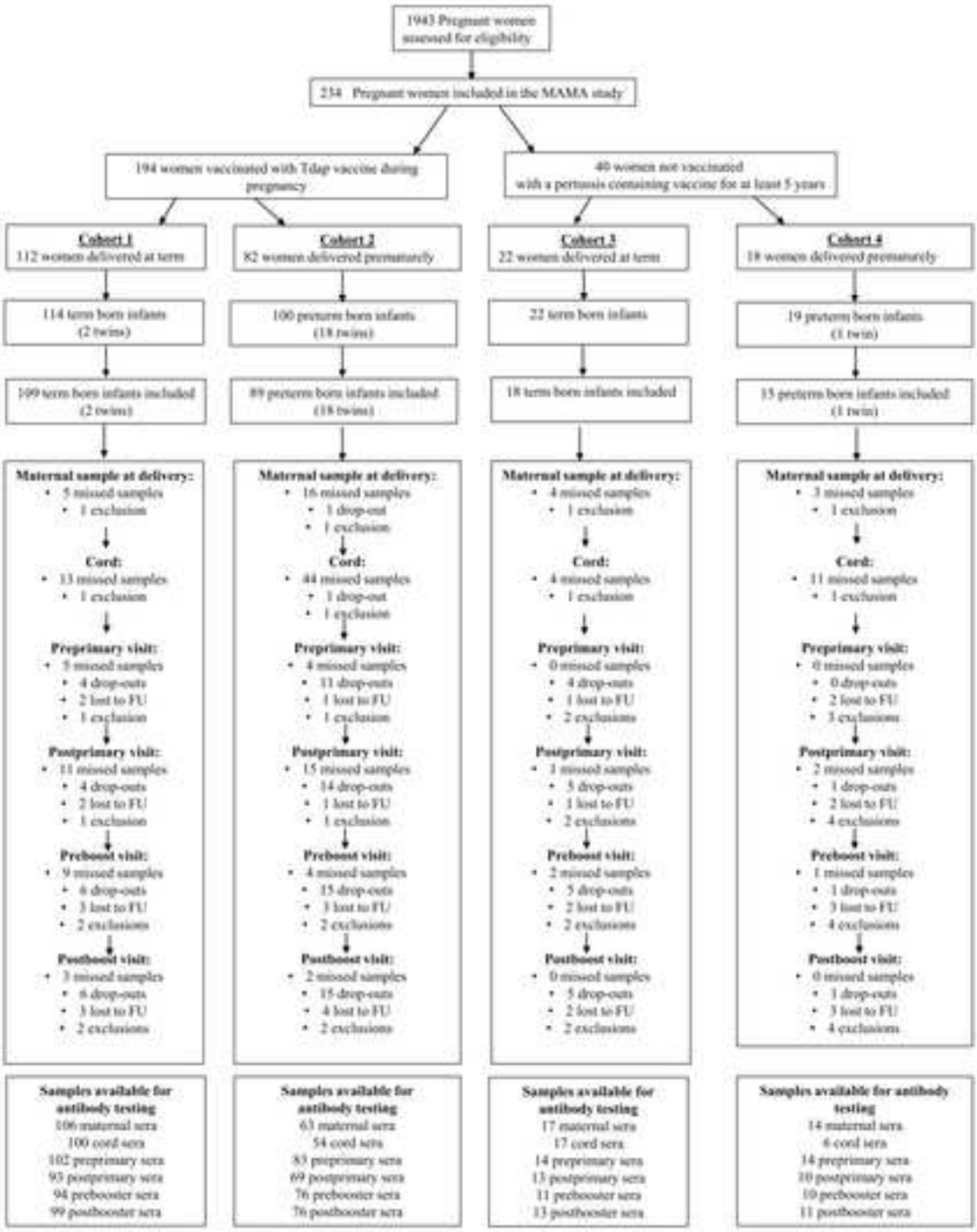

Supplementary Figure 2

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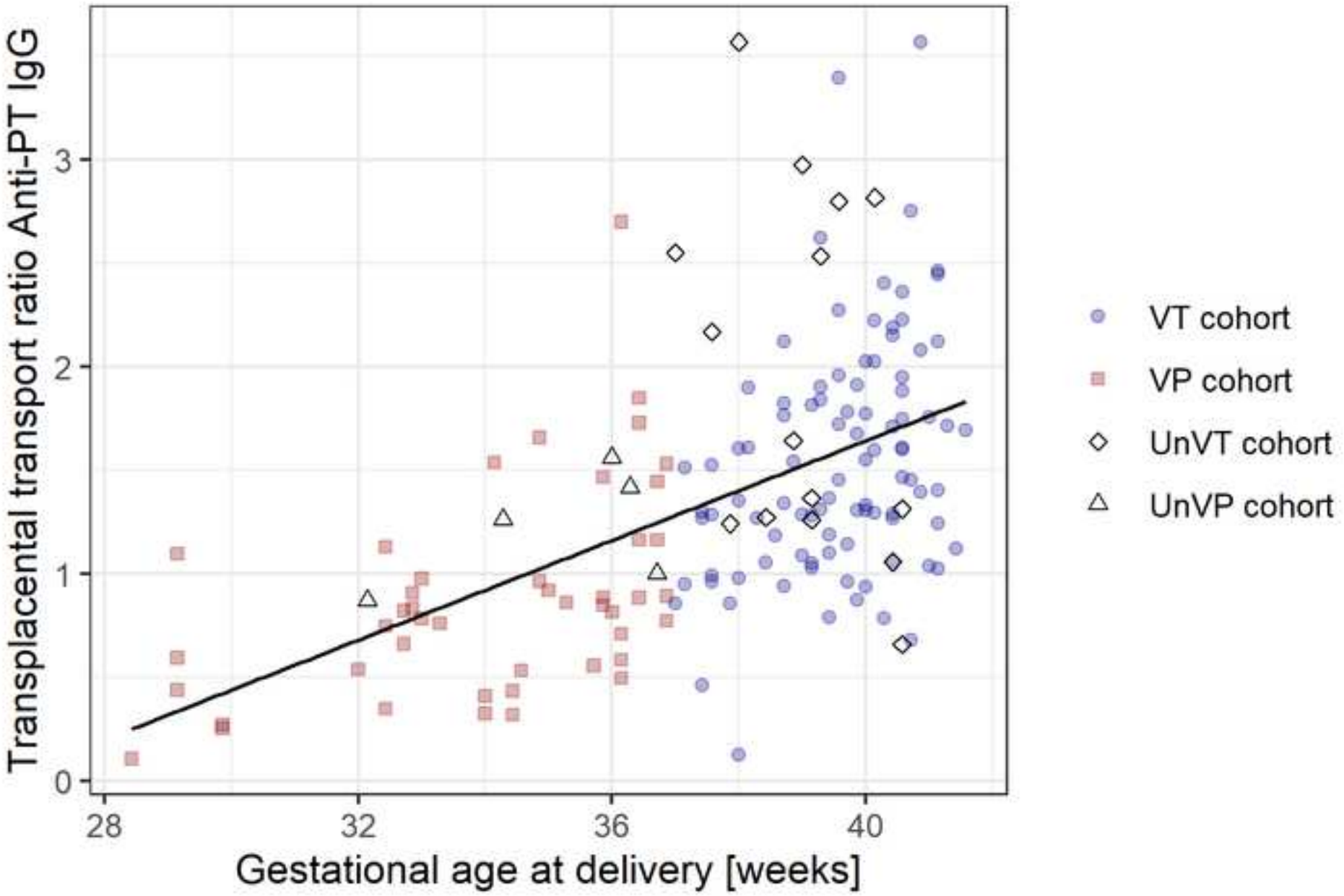

Supplementary Figure 3

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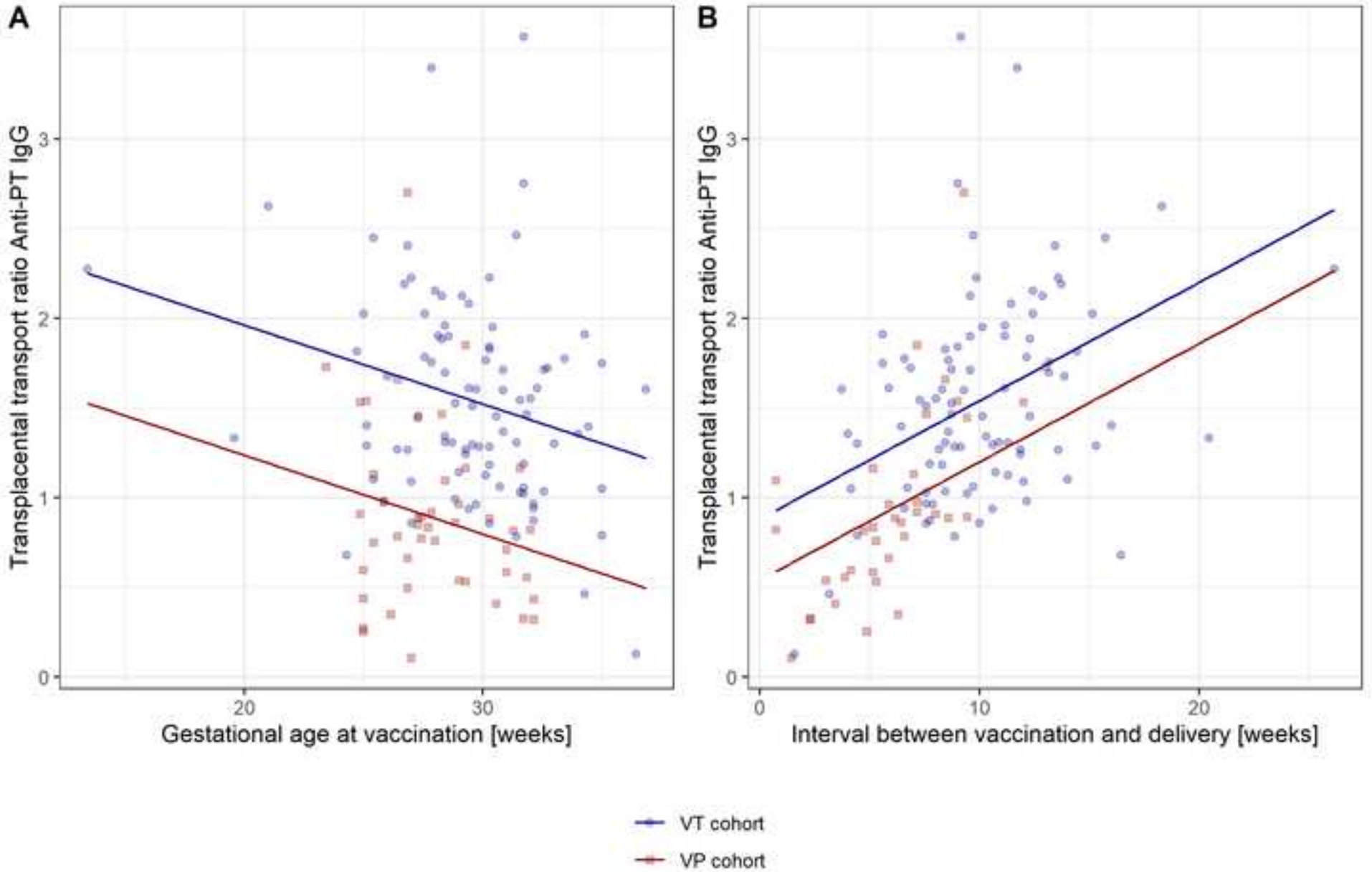

Supplementary Figure 4

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