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Breast Milk Antibody Levels in Tdap-Vaccinated Women After Preterm Delivery

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Background. Enrichment of breast milk (BM) with immunoglobulin (Ig) A and IgG through maternal vaccination could help infants combat targeted pathogens. However, evidence on this effect after preterm delivery is lacking. In this study, we investigated the total and anti-pertussis toxin (anti-PT)–specific IgA and IgG production in BM after term and preterm delivery in the presence of maternal Tdap (tetanus, diphtheria, acellular pertussis) vaccination.

Methods. Serum and BM samples of lactating women who delivered at term or prematurely and did or did not receive Tdap vaccine (Boostrix, GSK Biologicals) during pregnancy were collected as part of a clinical study (N = 234). Anti-PT IgA/IgG (IBL assay; Meso Scale Discovery assay) and total IgA/IgG (Thermofisher, on BM samples only) immunosorbent assays were performed on all samples collected at 72 hours and 4, 8, and 12 weeks postpartum.

Results. BM after preterm delivery contained anti-PT IgA and IgG geometric mean concentrations (GMCs) comparable to those after term delivery (eg, colostrum anti-PT IgA, 5.39 IU/mL vs 6.69 IU/mL, respectively). Maternal Tdap vaccination induced significantly higher anti-PT IgG GMCs in colostrum of vaccinated compared with unvaccinated women who delivered at term (0.110 IU/mL vs 0.027 IU/mL, $P = .009$). Anti-PT antibodies persisted up to 12 weeks postpartum.

Conclusions. This study provides evidence that maternal Tdap vaccination induces high Ig levels in BM after both term and preterm delivery and that these antibodies remain abundantly present throughout lactation, possibly offering additional mucosal protection during the most vulnerable period in early life.

Clinical Trial Registration. NCT02511327.

Keywords. maternal antibodies; maternal immunization; breast milk; preterm delivery; Tdap.

Human milk, an optimal source of nutrition, helps facilitate the neonate's transition from the maternal to the external environment, as it contains various components such as immunoglobulins (Igs), lactoferrin, hormones, cytokines, and immune cells [1–4]. These immunological factors, abundantly present in first milk (colostrum), exert major immunological functions such as protection against infectious diseases, prevention of inflammation, shaping microbiota, and stimulating the development of tissues [1–4]. Five to 14 days after delivery, this early immunologic milk changes to a mature milk, rich in lactose, fat, protein, and vitamins, fulfilling the nutritional needs of the growing newborn [5]. Although fewer immunologic components are secreted in mature milk, they remain present throughout lactation, continuously aiding development and promoting long-term health (eg, lower prevalence of diabetes, obesity, and high blood pressure) [2, 5–7].

Igs are one of the most important bioactive components in breast milk (BM), as they replenish the neonate's intestinal Ig levels and exert important functions such as opsonization of pathogens, selection of a favorable gut microbiota, and control of inflammation [1–3, 8–10]. These functions are especially beneficial in preterm infants who acquire smaller amounts of transplacental Igs compared with full-term infants [11] and suffer from various immunological immaturities [12]. The BM Ig levels and diversity are highly dependent on the stage of lactation [5] and the mother's previous exposure to pathogens and vaccination status [1, 3]. For example, pertussis vaccination during pregnancy is not only able to offer direct protection to the neonate via active transplacental transport of antibodies but also elevates the BM Ig levels, possibly offering additional mucosal immunity [4, 6, 9, 13, 14]. Evidence also suggests that preterm delivery itself induces changes in the BM Ig expression [2, 15], as higher levels of (total or secretory) IgA in colostrum [16–19] and mature milk [20–23] after preterm compared with term delivery were reported. This implies that delivering prematurely induces adaptive responses at the maternal site, promoting a higher excretion of protective BM

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Igs, which help overcome the immaturities of their immune system.

Unfortunately, data on BM composition after preterm delivery and the influences of maternal vaccination are lacking. To our knowledge, this study is the first to measure pertussis toxin (PT) specific IgA and IgG in serum and BM in a birth cohort of both Tdap (tetanus, diphtheria, acellular pertussis) vaccinated and unvaccinated lactating women who deliver either at term or prematurely. The relation between BM and serum antibodies was also investigated.

METHODS

Study Design

A prospective cohort study (N = 234: NCT02511327) conducted in Flanders, Belgium, recruited women before and soon after delivery who were either vaccinated or not vaccinated with a pertussis-containing vaccine (Tdap, Boostrix, GSK Biologicals) during pregnancy. Women suffering from mental illness, immunological disorders, or receiving experimental medication during pregnancy were excluded. Mother–infant pairs were assigned to 1 of the 4 cohorts based on vaccination status during pregnancy and gestational age (GA) at delivery (Figure 1A):

- VT cohort, *V*accinated women who delivered *T*erm (≥37 weeks GA); VP cohort, *V*accinated women who delivered *P*rematurely (24 to <37 weeks GA); UnVT cohort, *Un*Vaccinated women (no pertussis-containing vaccine in the last 5 years) who delivered *T*erm (≥37 weeks GA); or UnVP cohort, *Un*Vaccinated women (no pertussis-containing vaccine in the last 5 years) who delivered *P*rematurely (24 to <37 weeks GA) [24].

Here, only lactating women of the study were included. Vaccination was planned and performed within the regular healthcare system following the current Belgian recommendation, that is, between 24 and 32 GA. As part of these recommendations, postpartum vaccination (cocoon strategy) was also offered to women who did not receive Tdap vaccination during pregnancy. After obtaining informed consent, a first maternal blood (8 mL) and colostrum sample (5 mL) was collected within 72 hours after delivery. Additional BM and blood samples were collected from lactating women at 4, 8, and 12 weeks postpartum (Figure 1B). Parameters including the participant's demographics, medical history, drug use, method of BM sampling, last nursing session, and combined or exclusive breastfeeding at time of sampling were recorded. The University Hospital Antwerp Ethical Committee approved this study.

Storage of Samples

Collected whole BM samples (preserved in a refrigerator before pick-up) were aliquoted and stored in a −80°C freezer upon arrival. Serum of blood samples was aliquoted and stored at −35°C after centrifugation (1300 g, 10 minutes).

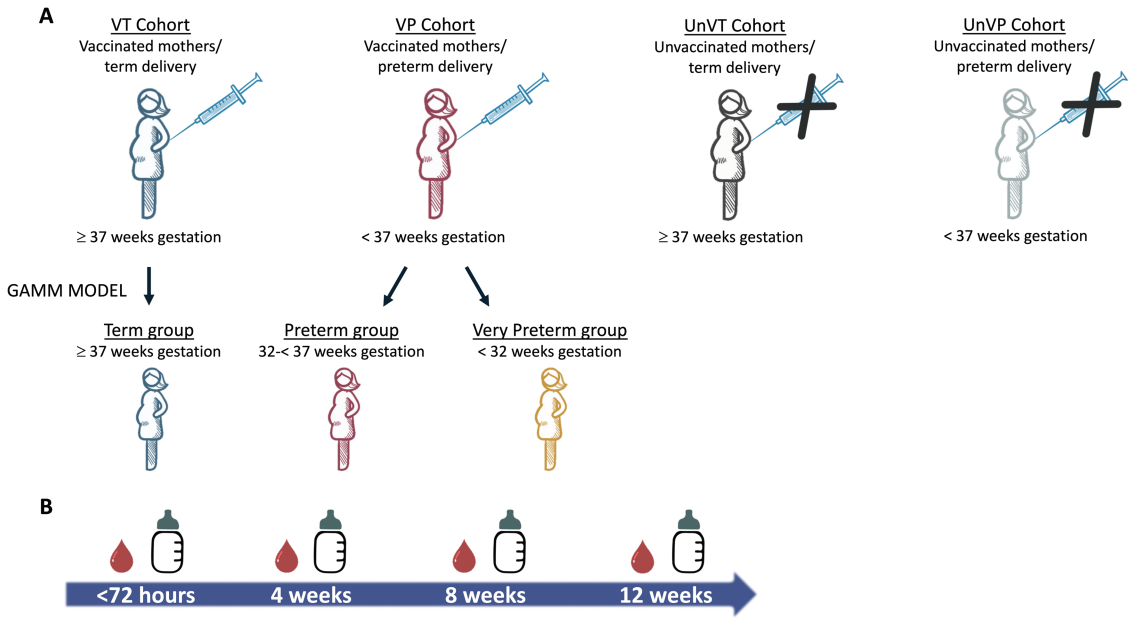

Figure 1. *A*. Schematic representation of the study design and the study groups included in the GAMM model. *B*, Postpartum maternal sample collection. Maternal vaccination was planned and performed within the regular healthcare system following the current Belgian recommendation, that is, between 24 and 32 weeks gestational age. Note: Postpartum vaccination (cocoon-strategy) was offered to women who did not receive Tdap (tetanus, diphtheria, acellular pertussis) vaccination during pregnancy. Abbreviations: GAMM, generalized additive mixed-effect model; UnVP, unvaccinated women with preterm born infants; UnVT, unvaccinated women with term born infants; VP, vaccinated women with preterm born infants; VT, vaccinated women with term born infants.

Detection of Pertussis Toxin–Specific Igs

Whole-BM samples were processed according to the protocol described by De Schutter et al [14]. After thawing the whole-BM samples, lipids and cells were removed following 2 centrifugation steps (1000 g for 10 minutes; 10 000 g for 30 minutes), and the aqueous fraction was collected. Following BM centrifugation and thawing of the serum samples, anti-PT–specific IgA and IgG antibodies were quantitatively measured in all samples using enzyme-linked immunosorbent assays (ELISA), reported in international units per milliliter (IU/mL). Total IgA and IgG antibodies (mg/mL; Thermofisher) were quantified in BM samples, and the anti-PT Ig/total Ig (IU/mg) ratios were calculated. Serum anti-PT IgG antibodies were cross-validated at the laboratory of Sanofi Pasteur using a diphtheria, tetanus, pertussis–electro-chemiluminescent Meso Scale Discovery assay (MSD; ELISA units/mL) [25]. A good correlation between both laboratory assays was found ($R^2 = 0.82$). The manufacturer's lower limits of detection (LLODs) for total IgA, total IgG, anti-PT IgA, and anti-PT IgG were 0.03 ng/mL, 0.24 ng/mL, 0.48 IU/mL, and 0.43 IU/mL (2 EU/mL; lower limits of quantification, MSD), respectively. IBL ELISA kits were validated for 1:100 diluted serum and plasma samples; therefore, the LLODs for BM anti-PT IgA and IgG were adjusted to accommodate the lower dilution factor (1:5 and 1:2 dilution, respectively).

Statistics

Demographic and clinical data were compared using χ^2 tests, parametric data were compared using the Student *t* test or paired Student *t* tests, and nonparametric data were compared using the Mann-Whitney *U* or Wilcoxon test where appropriate. Values below the LLOD were assigned the adapted LLOD/2. Ig values were natural log-transformed. Geometric mean concentrations (GMCs) with 95% confidence intervals (95% CIs) were computed for the log antibody concentrations.

The effect of premature delivery on BM was investigated by constructing 2 detailed models (see Supplementary Methods). Here, the unvaccinated cohorts were excluded due to the small sample size and because most unvaccinated mothers received postpartum Tdap vaccination, leading to potential bias. The vaccinated cohorts were subdivided into 3 groups: term (≥37 weeks GA), preterm (32 to <37 weeks GA), and very preterm (<32 weeks GA; Figure 1A), based on the World Health Organization and Global Alignment of Immunization Safety Assessment in Pregnancy definitions [26, 27].

Statistics were performed using statistical software JMPpro version 14, and R (version 3.6.2, package mgcv for the generalized additive mixed-effect models [GAMMs]) was used for the models. Cutoff for statistical significance was set at 5%, without correction for multiple testing. Sample size calculation was not performed; all samples were convenience samples.

RESULTS

Demographics

In total, 543 BM and 597 serum samples of 177 lactating women were available for analysis (N = 87 VT, N = 63 VP, N = 15 UnVT, N = 12 UnVP cohort; Supplementary Figure 1). For the model-based analysis, 463 BM and 505 serum samples were used (N = 87 term, N = 49 preterm, N = 14 very preterm group).

The demographic characteristics are presented in Table 1 and were comparable to those of the entire study population (Pertussis Immunization during pregnancy: Assessment of the role of maternal antibodies on immune responses in term and preterm-born infants). Expected demographic differences between the cohorts caused by premature delivery were observed (GA at delivery, interval between maternal vaccination and delivery, and mode of delivery).

The majority of pregnant women (86.7%) were vaccinated within the recommended GA period of 24–32 weeks; 61.0% of the unvaccinated lactating mothers received postpartum vaccination, of which 82.0% within 72 hours after delivery.

BM Antibodies

Comparable anti-PT IgA levels were observed in all cohorts at the distinct time points (Supplementary Table 1A). However, for anti-PT IgG, significantly higher antibody levels were observed in colostrum of the VT cohort compared with the UnVT cohort (GMC, 0.110 IU/mL vs 0.027 IU/mL, $P = .009$). The same trend (not significant) was detected between the VP and UnVP cohort. At all other time points, anti-PT IgG levels were comparable between all 4 cohorts (Supplementary Table 1B).

Measurements of total IgA in BM showed no differences between the cohorts at any point in time. This was similar for total IgG in colostrum at 8 and 12 weeks; however, at 4 weeks, significantly higher total IgG levels were detected in women who delivered at term compared with women who delivered prematurely (VT vs VP cohort, $P = .009$; UnVT vs UnVP cohort, $P = .038$; Figure 2C, 2D).

The colostrum IgA ratios (anti-PT Ig/total Ig [IU/mg]) of vaccinated women who delivered prematurely were significantly lower compared with their term counterparts (VT vs VP, $P = .039$; Figure 2E and F). Significantly higher colostrum IgG ratios were reported in all vaccinated women compared with unvaccinated women (VT vs UnVT, $P = .007$; VP vs UnVP, $P = .014$).

Serum Antibodies of Lactating Women

Maternal vaccination induced higher serum anti-PT IgA and IgG levels in vaccinated mothers (VT and VP cohort) compared with unvaccinated mothers (UnVT and UnVP cohort) at delivery (Supplementary Figure 2). For anti-PT IgG, the antibody levels of in-pregnancy vaccinated mothers remained higher compared with those of unvaccinated mothers up until 12 weeks postpartum (significant for VT vs UnVT at 4 and

Table 1. Demographics of the Lactating Mothers

	VT Cohort	VP Cohort			UnVT Cohort	UnVP Cohort
Groups of the Model-based Analysis	Term Group (≥37 Weeks GA)	Total Preterm Group (<37 Weeks GA)	Preterm Group (32 to < 37 Weeks GA)	Very Preterm Group (< 32 Weeks GA)	NA	NA
Lactating women, N	87	63	49	14	15	12
Mean maternal age at delivery (SD), years	31.3 (3.7)	30.6 (3.9)	30.7 (3.9)	30.2 (4.0)	31.3 (3.0)	31.6 (4.7)
Ethnicity, N (%)						
Caucasian	81 (93.1)	62 (98.4)	48 (98.0)	14 (100.0)	13 (86.7)	10 (83.3)
Non-caucasian	6 (6.9)	1 (1.6)	1 (2.0)	0 (0.0)	2 (13.3)	2 (16.7)
Median GA at delivery (min-max), weeks	39.7 (37.0–41.4)	34.4 (28.4–36.9)	34.9 (32–36.9)	29.8 (28.4–31.9)	39.3 (37.6–40.7)	31.7 (25.7–36.7)
Median GA at vaccination (min-max), weeks	29.4 (16.9–36.4)	28.3 (23.4–35.9)	28.4 (23.4–35.9)	27.3 (25.0–29.7)	NA	NA
Interval between maternal vaccination and delivery (SD), weeks	10.5 (3.7)	5.3 (3.0)	6.1 (2.9)	2.6 (1.6)	NA	NA
Trimester of vaccination, N (%)					NA	NA
Second	27 (31.0)	28 (44.4)	20 (40.8)	8 (57.1)		
Third	60 (69.0)	35 (55.6)	29 (59.2)	6 (42.9)		
Parity, N (%)						
Uniparous	50 (57.5)	45 (71.4)	34 (69.4)	11 (78.6)	8 (53.3)	7 (58.3)
Multiparous	37 (42.5)	18 (28.6)	15 (30.6)	3 (21.4)	7 (46.7)	5 (41.7)
Mode of delivery, N (%)						
Vaginal	66 (75.9)	37 (58.7)	29 (59.2)	8 (57.1)	10 (66.7)	8 (66.7)
Cesarean section	21 (24.1)	26 (41.3)	20 (40.8)	6 (42.9)	5 (33.3)	4 (33.3)
Median interval between nursing and sampling (min-max), hours						
Colostrum	1.0 (0.0–5.5)	2.5 (0.0–19)	2.5 (0.0–19.0)	2.5 (0.2–3.0)	0.7 (0.2–5.8)	1.2 (0.5–6.0)
Week 4	1.3 (0.0–23.7)	3.0 (0.0–20.8)	2.8 (0.0–18.0)	3.1 (1.9–20.8)	0.75 (0.2–7.3)	2.75 (0.2–4.5)
Week 8	1.5 (0.0–14.0)	2.5 (0.0–23.0)	2.5 (0.0–23.0)	4.8 (0.5–14.3)	1.8 (0.0–11.0)	3.5 (0.5–14.8)
Week 12	2.4 (0.0–23.0)	2.4 (0.0–48.5)	2.1 (0.0–48.5)	4.5 (0.5–10.3)	2.2 (0.0–10.5)	3.5 (0.5–24)

Abbreviations: GA, gestational age; min-max, minimum-maximum; NA, not applicable; SD, standard deviation; UnVP, unvaccinated women with preterm born infants; UnVT, unvaccinated women with term born infants; VP, vaccinated women with preterm born infants; VT, vaccinated women with term born infants.

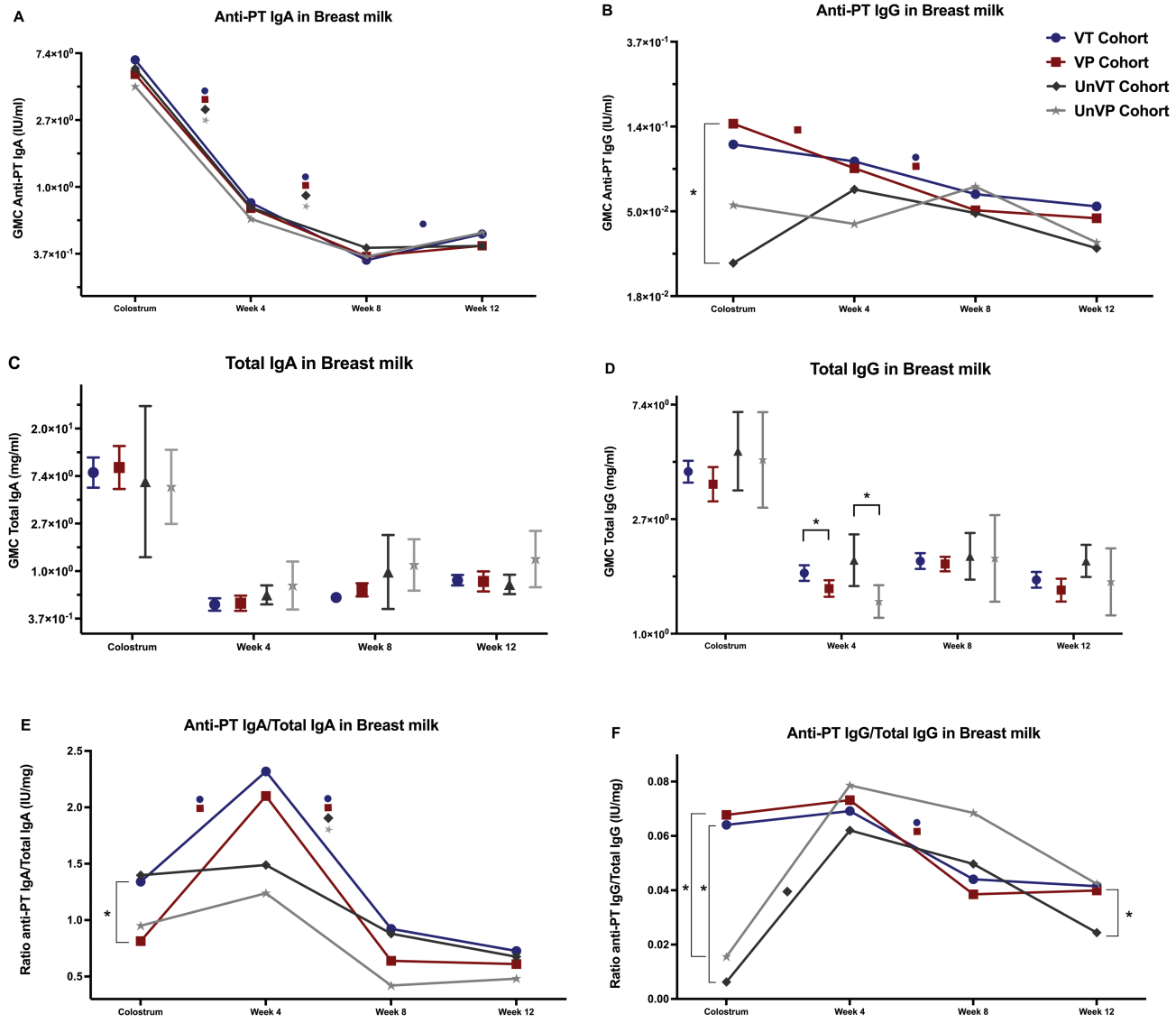

Figure 2. Representation of the anti-PT IgA *(A)*, anti-PT IgG *(B)*, total IgA *(C)*, and total IgG *(D)* geometric mean concentration (95% confidence interval) in breast milk, together with the anti-PT IgA/total IgA *(E)* and anti-PT IgG/total IgG ratios *(F)*. * indicates significant differences between cohorts; ●■◆★ indicates significant antibody decay for the VT, VP, UnVT, and UnVP cohorts, respectively. Abbreviations: GMC, geometric mean concentration; Ig, immunoglobulin; PT, pertussis toxin; UnVP, unvaccinated women with preterm born infants; UnVT, unvaccinated women with term born infants; VP, vaccinated women with preterm born infants; VT, vaccinated women with term born infants

12 weeks, $P = .020$ and .021, respectively). However, comparing postpartum vaccinated to in-pregnancy vaccinated mothers showed no significant differences from 4 weeks onward (data not shown).

For anti-PT IgA, significantly higher levels at 4 and 8 weeks postpartum were detected in the unvaccinated cohorts compared with the vaccinated cohorts (only significant for VT vs UnVT, $P = .009$ and .017, respectively). At 12 weeks postpartum, higher anti-PT IgA levels were again detected in the vaccinated cohorts (VT vs UnVT, $P = .0330$). A comparison between in-pregnancy vaccinated and postpartum vaccinated mothers showed no differences from 8 weeks onward (data not shown).

Some differences between women who delivered at term or prematurely were also detected at 4 and 8 weeks postpartum, as significantly higher anti-PT IgA levels were observed in VP compared with VT mothers ($P < .001$ and .006, respectively). At 4 weeks postpartum, the reverse was observed for anti-PT IgG, as the VT cohort had significantly higher antibody levels compared with the VP cohort ($P = .041$).

Model-based Analysis of Lactating Women Who Received Tdap During Pregnancy

Relation Between Antibodies in BM and Serum

With an exception at 8 weeks postpartum ($P = .040$; positive relation), no significant linear relation was observed between

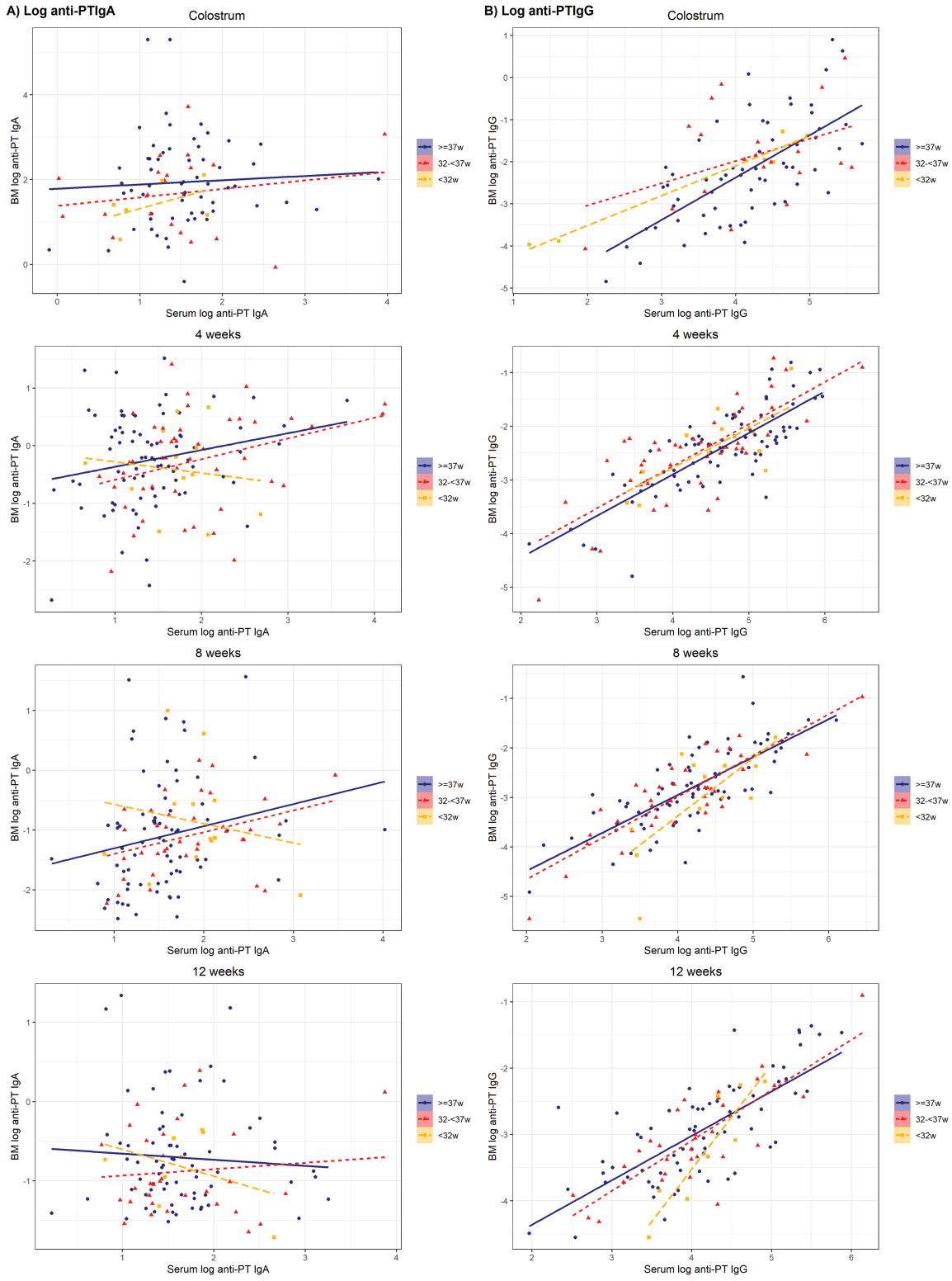

Figure 3. Linear model of log anti-PT IgA *(A)* and log anti-PT IgG *(B)* in breast milk vs in serum of the term, preterm, and very preterm groups according to the postpartum sample collection time point. Abbreviations: BM, breast milk; Ig, immunoglobulin; PT, pertussis toxin.

anti-PT IgA in BM and serum at any of the time points (Figure 3A). In contrast, anti-PT IgG in BM showed a significant positive relation with serum antibodies at each time point for each of the groups ($P < .001$; Figure 3B). At all time points the slopes for the groups were comparable, except for the slope of the very preterm group at 12 weeks postpartum, which increased

significantly faster (more anti-PT IgG antibodies in serum lead to more anti-PT IgG antibodies in BM) than the slope of their term counterparts ($P = .017$). Overall, the relation between serum and BM anti-PT IgA antibodies was weak (<7% variability) and was stronger for anti-PT IgG (43%–62% variability, depending on the time point).

Antibody Decay in BM Over Time

The final multivariate GAMMs included all predetermined variables (see Supplementary Methods) for all 4 antibody outcomes (anti-PT IgA, anti-PT IgG, ratio IgA, and ratio IgG), no collinearity was observed between the variables, and no interaction terms were included. The estimated antibody decay over time for all 4 outcomes for each group was significant for all BM antibody concentrations ($P < .001$; Figure 4). For the anti-PT and ratio IgA models (Figure 4A, 4B), the overall estimated trajectories were not significantly different across the groups ($P = .628$ and 1.000, respectively). The interval between the last feeding and sampling in hours had a significant positive influence on the anti-PT IgA in BM ($P = .011$), indicating that antibodies in BM increased as the interval grew. This was not observed for ratio IgA, where no significant influences were observed for any of the variables (except time). However, for anti-PT and ratio IgG, the decay pattern significantly differed across the groups ($P = .005$ and .008, respectively; Figure 4C, D), as the preterm group had a significantly higher level of anti-PT and ratio IgG in colostrum ($P = .014$ and .004, respectively) compared with the term group (not observed for the very preterm group). Additionally, mothers with higher serum anti-PT and ratio IgG antibodies had higher BM anti-PT and ratio IgG antibodies ($P < .001$). Approximately 29%–78% of the variability in the BM antibodies (depending on antibody type) was described by these GAMM models.

DISCUSSION

We report, for the first time, comparable anti-PT IgA and IgG levels in BM of in-pregnancy vaccinated mothers after both term and preterm delivery and provide confidence that maternal Tdap vaccination can be beneficial for the preterm infant.

Vaccination of pregnant women within the Belgian healthcare system reached a coverage of 69.3% in 2016 [28], complicating the recruitment of unvaccinated mothers. Despite this hurdle, Tdap vaccination during pregnancy was associated with higher (but not statistically different) anti-PT IgA levels in colostrum of vaccinated mothers, similar to the study by Abu Raya et al [13]. Higher colostrum anti-PT IgG levels were also observed in the vaccinated mothers and were significantly different between the term cohorts (VT vs UnVT), confirming the evidence that vaccination during pregnancy elevates the BM Ig levels [13, 14]. From 4 weeks onward, the BM anti-PT IgG levels were comparable in all 4 cohorts, as high uptake of postpartum Tdap vaccination is known to induce high IgG antibody titers in BM within 2 weeks after vaccination [29].

The overall benefit of Tdap vaccination during pregnancy for premature infants remains unclear. However, a recent study showed that extending the window of maternal

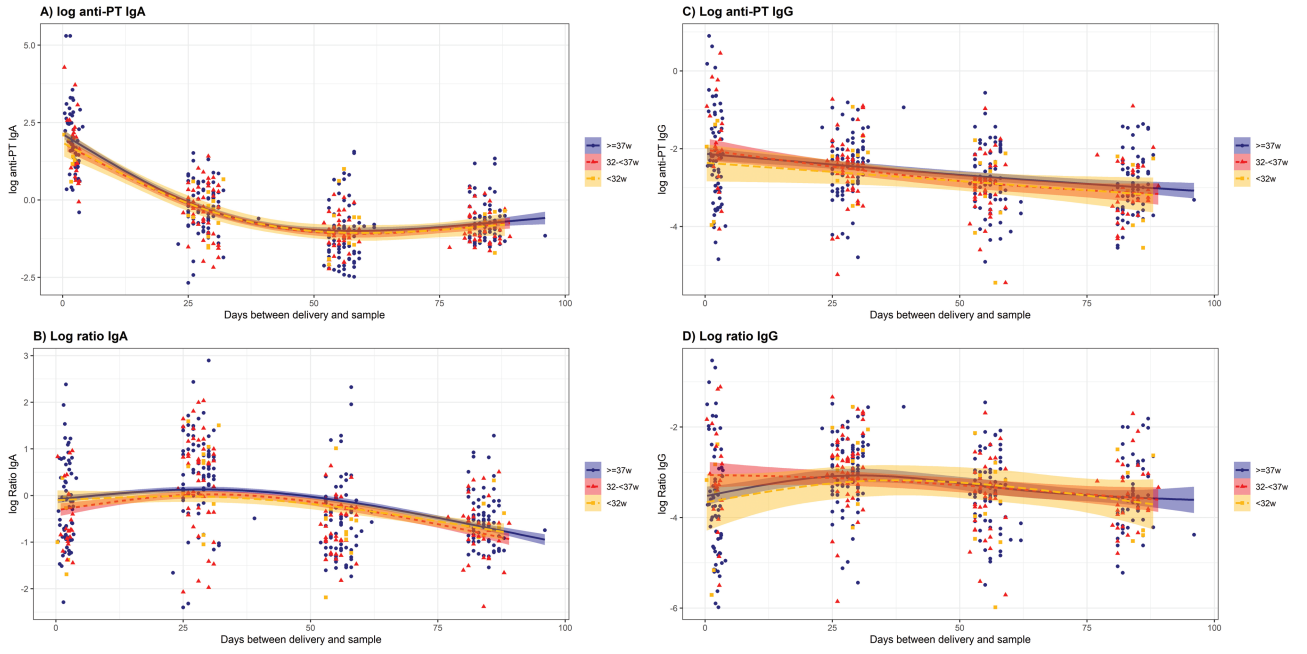

Figure 4. BM Antibody outcomes over time, adapted according to predefined variables using multivariate generalized additive mixed-effect model. Abbreviations: Ig, immunoglobulin; PT, pertussis toxin.

vaccination from the early third (28–32 weeks of gestation) to the second trimester (20–32 weeks of gestation) reduced the number of preterm infants hospitalized with pertussis disease in England [30]. Our study demonstrates that maternal vaccination (and, to a lesser extent, postpartum vaccination) induces comparable anti-PT antibody levels in BM after both term and preterm delivery. These BM Igs could help bridge the vulnerability gap induced by the shortened period of transplacental transport of antibodies linked to prematurity by potentially offering additional mucosal protection. Other observations, such as high anti-PT IgA and IgG ratios in BM at 4 weeks postpartum and the persistence of these antibodies 12 weeks after delivery, strengthens the hypothesis that maternal vaccination induces beneficial changes in the milk composition after both term and preterm delivery. These observations were again confirmed after GAMM modeling of the term, preterm, and very preterm groups of vaccinated women. Within these models, we also show that BM anti-PT IgA antibodies accumulate in between feeding moments, similar to the study by De Schutter et al [14], and that a positive linear relationship exists between BM and serum anti-PT IgG (not anti-PT IgA).

However, some differences in the BM composition of mothers who delivered term or prematurely were reported. We observed significantly lower IgA ratios in colostrum of the VP cohort compared with the VT cohort, which could have been the result of a slightly higher expression of total IgA in BM after preterm delivery that is promoted by the activation of adaptive responses that occur at the maternal site following preterm birth [16–23]. In contradiction, significantly lower total IgG levels were detected 4 weeks postpartum in BM of mothers who delivered prematurely compared with those who delivered at term, suggesting that this compensatory mechanism might not be present for IgG or is only present during early lactation. The latter was observed with the GAMM model, as the preterm group (GA: 32 to <37 weeks) had significantly higher levels of anti-PT IgG and ratio IgG in colostrum compared with the term and very preterm group. This observation also suggests that the composition of Ig is highly dependent on gestational length, confirming the study by Castellote et al [16] who reported the lack of this compensatory mechanism after very preterm delivery (<30 weeks of gestation).

Our study has several limitations. First, during the hospital recruitment visits, more women with high-risk pregnancies were approached, possibly resulting in a selection bias for the term cohorts and lower comparability with the real-life situation. In addition, the high level of compliance with postpartum cocoon vaccination in the unvaccinated cohorts made a fair comparison with the vaccinated cohorts difficult from 4 weeks postpartum onward. Second, our study would have benefited from an additional sampling time point during the transitional milk phase (3 days to 2 weeks after delivery) to better map the BM changes that occur within the first weeks. Nevertheless, we were able to collect a large amount of BM samples at all time points and observe a similar decay pattern for anti-PT IgA, as described in the study by Abu Raya et al [13]. Last, a note of caution is due when interpreting these results, as the samples were a convenience sample and no power calculation nor correction for multiple testing were performed.

CONCLUSIONS

Tdap vaccination during pregnancy is capable of inducing high anti-PT IgA and IgG antibody levels in colostrum and throughout later lactation in women who deliver term and preterm babies. In addition, delivering between 32 and <37 weeks might trigger a compensatory maternal response, increasing the amount of Igs during the early days of lactation. This early lactation IgG enrichment together with the help of maternal vaccination aids to replenish the preterm's Ig shortage at the mucosal level and possibly offers additional protection against pathogens in early life. Nevertheless, more research is needed because the extent of this added protective mucosal effect of BM needs to be proven.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. P. V.D. reports grants from GSK Biologicals, Pfizer, Sanofi, Merck Takeda, Baxter, CanSino China, Themis, Osivax, J&J, and Abbott (the University of Antwerp obtains grants from several Small and Medium Enterprises (SME) and vaccine manufacturers for the conduct of vaccine trials for which P. V.D. is the investigator and for the support of the Viral Hepatitis Prevention Board (P. V.D. obtains no personal remuneration)) and grants from the Bill & Melinda Gates Foundation, PATH, the Flemish government, and the European Union (EU; the University of Antwerp obtains grants from foundations, the EU, and the Flemish government for the conduct of trials and vaccine research for which P. V.D. is the principal investigator) outside the submitted works. All remaining authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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