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Pertussis vaccination during pregnancy in Belgium: results of a prospective controlled cohort study

Running title: Pertussis vaccination during pregnancy

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Abstract

Vaccination during pregnancy has been recommended in some countries as a means to protect young infants from severe infection. Nevertheless, many aspects are still unknown and possible blunting of the infant's immune responses by maternal antibodies, is one of the concerns with maternal vaccination. We report the first prospective controlled cohort study in women and infants on the effects of using Boostrix®, a combined tetanus, diphtheria and acellular pertussis vaccine, during pregnancy. The primary aim was to measure the influence of this booster dose on the titer and duration of the presence of maternal antibodies in the infants and assess possible interference with infant immune responses.

In a controlled cohort study, 57 pregnant women were vaccinated with Tdap vaccine (Tetanus Diphtheria acellular Pertussis, Boostrix, GSK Biologicals), at a mean gestational age of 28.6 weeks. A control group of pregnant women (N=42) received no vaccine. Antibody geometric mean concentrations (GMCs) against tetanus (TT), diphtheria (DT), pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) were measured with commercial ELISA tests in samples taken preceding maternal vaccination and one month afterwards, at delivery and from the cord blood, and in infants before and 1 month after the primary series of 3 pertussis containing hexavalent vaccines.

Infants born to vaccinated women had significantly higher GMC at birth and during the first 2 months of life for all vaccine antigens compared to the offspring of unvaccinated women, thereby closing the susceptibility gap for pertussis in infants. However, blunting was noticed for

infant diphtheria and pertussis toxin vaccine responses ($p<0.001$) in the infants from vaccinated women after the primary vaccination schedule (week 8-12 and 16).

Since pertussis vaccination has been recommended during pregnancy already, the results of this study support that recommendation and provide additional scientific evidence to document possible interference by maternal antibodies.

Clinicaltrials.gov identifier: NCT01698346

Key words: pertussis, vaccination in pregnancy, maternal antibodies, blunting

Introduction

Pertussis, caused by *Bordetella pertussis*, is a highly contagious respiratory illness and a major cause of infant morbidity and mortality. Global pertussis vaccination programs have been introduced with success and approximately 86% of infants worldwide have received 3 doses of the diphtheria-tetanus-pertussis (DTP3) vaccine [1].

However, a decade after the switch from the whole-cell (wP) vaccine to the acellular pertussis (aP) vaccine, a cyclic resurgence has been reported in several industrialized countries. The reason is presumed to be multifactorial, with waning immunity after the primary or booster vaccination as the primary cause. A resurgence has been observed in all age categories; however, severe morbidity and mortality occurs primarily in young infants who are not fully vaccinated [2, 3]. The majority of cases are found in adolescents and adults, due to waning immunity [4], and these populations represent sources of infection for young infants.

In Belgium, pertussis vaccination with a hexavalent aP-containing vaccine is offered at 8, 12, and 16 weeks and 15 months of age. Booster doses for children 4-6 years of age (since 2004) and for adolescents 14-16 years of age have been recommended since 2009. Additionally, receiving a booster dose once during adulthood has been recommended since 2013 [5]. Nevertheless, the total number of confirmed cases increased in Belgium from 93 in 2005 to 843 cases in 2013 [6], of which many (25.4% in 2013) were found in infants under the age of 1 year.

Partial primary protection against infectious diseases is offered at birth by maternal immunoglobulin G (IgG) antibodies [7, 8], with an estimated half- life of 6 weeks for pertussis [8]. The amount of transmitted antibodies depends on the placental function and the

concentration of maternal antibodies in the pregnant woman [9]. The latter depends on the time lapse since the last vaccination or infection [10] and the titer of passively transmitted pertussis maternal antibodies is often low [11]. Thus, increasing the load of maternal antibodies by vaccination during pregnancy is, with the currently available vaccines, the only way to offer passive protection to the newborn at birth [12]. During the first weeks of life, these maternal antibodies disappear in the newborn due to natural clearance [9, 13].

Vaccination during pregnancy is recommended in an increasing number of countries (e.g. UK, USA, Belgium, New Zealand, etc). Research has been performed on the immunological and safety aspects of the strategy [14-18]; nevertheless, many aspects are still unknown, and the possible interference of maternal antibodies with the infant's immune responses is one of the concerns.

To the best of our knowledge, no other data have been published on the effects of using the combined tetanus, diphtheria and acellular pertussis vaccine Boostrix® (GSK, Rixensart, Belgium) during pregnancy. The primary aim was to measure the influence of this booster vaccination on the titer and the duration of maternal antibodies in infants and to assess possible interference.

Material and methods

A prospective controlled cohort study was conducted in accordance with the Declaration of Helsinki, ICH-GCP, and the procedures established by Belgian law and was approved by the ethics committee of the University of Antwerp, Belgium (Clinicaltrials.gov identifier: NCT01698346). Written informed consent was obtained from all participants and from both parents of the participating infants (in accordance with the Belgian law and IRB regulations).

Healthy pregnant women and their healthy offspring from 5 different hospitals in the province of Antwerp, Belgium, were included in the study, and follow-up remains ongoing. Pregnant women were included in either a vaccine group, receiving an acellular pertussis vaccine, or a control group, if they had not received any pertussis-containing vaccine for at least 10 years. Strict randomization was not possible because some women were advised positively or negatively by their treating physician on the pertussis vaccination in pregnancy and were included accordingly. The recommendation for receiving the pertussis vaccination during pregnancy by the Belgian National Immunization Technical Advisory Group (NITAG, since August 2013) was not yet in place during the recruitment phase of this study, only a recommendation for cocoon vaccination. However, by 2012, the VVOG (Association of Flemish Obstetricians and Gynecologists) had recommended the ACIP as a valuable alternative for cocoon vaccination on its website. This recommendation was followed by some Belgian clinicians.

Strict inclusion and exclusion criteria were used (Annex 1).

An extended questionnaire collected information on obstetrical risk factors, demographics, a general vaccination and pertussis-specific history, and a general medical history. Growth parameters, breastfeeding data, day-care attendance, immunization data, and medical histories for all household members were collected at each visit.

Study vaccines

Licensed Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) was used to immunize pregnant women. Boostrix® contains 5 Lf of tetanus toxoid (TT), 2.5 Lf of diphtheria toxoid (DT), 8 mcg of inactivated pertussis toxoid (PT), 8 mcg of filamentous haemaglutinin (FHA), and 2.5 mcg of pertactin (Prn). Infants were vaccinated with a hexavalent vaccine (Infanrix hexa®, GSK Biologicals, Rixensart, Belgium). Infanrix hexa® contains 25 Lf of DT, 10 Lf of TT, 25 mcg PT, 25 mcg FHA and 8 mcg Prn, inactivated poliovirus, hepatitis B surface antigens and *Haemophilus influenzae* type B polysaccharide.

Study procedures

Venous blood (10 cc) was collected from all participating women immediately preceding the vaccination, at 1 month (28-31 days) after vaccination, and at delivery. The maternal vaccination was performed by the study physician or study nurse under supervision. Cord blood was collected at delivery (10 cc). Blood samples (2 cc) were collected from the infants before starting the primary schedule (week 8 ± 4days) and at month 5 (28-35 days after the third vaccine dose). Infant vaccines were administered in the regular health care system at the well-baby clinics or a pediatrician. Further follow-up is ongoing, with blood samples being collected before and after

an Infanrix hexa® booster dose is given at month 15 (data not shown). The samples were centrifuged at 2000 rpm within 24 hours and stored at -20°C.

Safety assessments

Systemic reactions were monitored by a medical doctor in all women for 30 minutes post-vaccination. Adverse events were monitored for 30 days post-vaccination and included: injection site pain, swelling, erythema, and general symptoms such as myalgia and fever. Serious adverse events during the pregnancy and follow-up period were documented. Whether an adverse was caused by the immunization was judged by the investigators who considered temporality, biologic plausibility, as well as the identification of alternative etiologies for each event. Possible congenital abnormalities were also monitored in the offspring.

Laboratory

All samples were tested with commercially available ELISA kits at the National Reference Centre for Bordetella. The Virion/Serion® kit (ANL, Copenhagen) was used to detect anti-PT IgG antibodies, and the EuroImmune® ELISA kit was used to detect anti-FHA and anti-Prn IgG antibodies. Anti-TT and anti-DT IgG antibodies were detected using the Virotech/Sekisui® ELISA. Serum samples were tested in duplicate at a dilution of 1:100 (PT, TT and DT), 1:400 (FHA) and 1:800 (Prn). All OD results were converted into international units per milliliter (IU/mL). For tetanus and diphtheria, the limits of detection were 0.01 IU/mL and 0.03 IU/mL, respectively. All titers are expressed in International Units IU/ml , using respective WHO standards (NIBSC 06/140 for pertussis, NIBSC code TE-3 for tetanus and NIBSC 00/496 for diphtheria). For Pertussis these

144 international units are equivalent to the CBER EU units of FDA (A. van der Zee et al, Clin Microbiol Rev
145 28: 1005-1026, 2015)).

146 An international independent validation was performed to guarantuee the reliability of the
147 results. A random selection of samples (N=177) was reanalyzed at the Canadian Center for
148 Vaccinology in Halifax, where CBER equivalent sera based on the WHO standard lot number 3 were
149 used. A positive correlation was found in the results from both laboratories. The protective
150 threshold of antibodies (a correlate of protection) is not known for pertussis [19]. For tetanus
151 and diphtheria, a correlate of protection is defined as 0.1 IU/mL for tetanus and 0.01-0.1 IU/mL
152 for diphtheria.

153 Statistics

154 A sample size calculation was performed, based on previous results [20]. Accordingly, a
155 population of 50 subjects in both study arms would be sufficient to detect significant differences
156 in antibody titers at several time points.

157 Statistical tests included parametric tests: (paired) t-tests and chi-square tests and their non-
158 parametric alternatives: (paired) Wilcoxon tests and Fisher Exact tests whenever the underlying
159 assumptions of the parameteric tests were violated, ie normality and sparseness, respectively
160 [21, 22].

161 The presence of twins in the data resulted in using different sample sizes for outcomes related
162 to women and children. Data were assumed to be missing completely at random and complete
163 case analyses were conducted rendering unbiased estimates though at the cost of a loss of

efficiency [23]. The loss of efficiency resulting from excluding observations was limited due to the limited amount of missing data (12.4%).

Assessing the impact of the different mother and child characteristics on GMC for each of the different time points was done using a regression approach with outcome the log titer values, symmetrizing the response, and consisting of three consecutive steps: (1) variable selection using random forests [24] (Annex 2); (2) backward model selection using multiple linear regression based on AIC; and (3) further model reduction using likelihood ratio tests [22]. This model building procedure was used before [25].

Blunting of vaccine immune responses among infants was defined as a lesser GMC of specific IgG antibodies at a certain point in time in the offspring of the vaccinated women compared to the titer in the offspring of the control group.

Results

General characteristics of the study population (Table 1)

In total, 57 healthy pregnant women were vaccinated (the vaccine group), and 42 women were identified as controls (the control group). The children were born between April 2, 2012 and April 16, 2014. Blood samples were taken between February 6, 2012 and September 18, 2014. The mean interval between the Tdap immunization and delivery was 77.1 days (39-117 days). The mean gestational age at vaccination was 28.6 (22-33) weeks. After delivery, 55 children were included in the vaccine group (including 2 sets of twins) and 26 children in the control group. Reasons for exclusion included: premature delivery (N=1), children not vaccinated according to protocol (N=2), and the failure to obtain informed consent signed by both parents (N=17). Additionally, 2 children from the control group were excluded after the blood sample on week 8 due to delayed primary vaccination. No significant differences in demographics were found between both groups. The clinical history performed at every visit did not identify any clinical case of pertussis.

Safety results *(Table 2)*

Of the 57 women in the vaccine group, 50 adverse events (AE) were reported in 46 women. Most symptoms were mild and self-limited and were resolved within 72 hours after vaccination. Stiffness of the arm at the injection site was the most commonly reported AE (N=42), followed by minor swelling at the injection spot. Five AE (vaginal thrush, reflux, fever <38.5°C, extensive limb swelling, and rashes on the abdomen and arms) required the use of concomitant

196 medication. Fever was described in only 1 vaccinated women (1.75%). The mean duration of all
197 AE was 2.30 days (1-10 days).

198 A total of 11 serious adverse events (SAE) were reported in the vaccine group; none of these
199 SAE were related to the vaccination (according to the investigator's opinion). In the control
200 group, 3 SAE were reported. The reported SAE included 1 case of preterm preeclampsia, 4 of
201 term preeclampsias, 4 of premature contractions, 3 of hypertension, 1 of oligohydramnios and 1
202 of placenta previa.

203 In total, 8 SAE requiring hospitalization for at least 1 hour, were reported in the infants: 7 in the
204 vaccine group and 1 in the control group. The mean duration of the SAE was 7.75 days (1-31
205 days). The reported SAE included: 1 premature delivery, 1 fever at birth, 1 hypoglycemia at
206 birth, 1 pneumonia at birth, 2 infections that required hospitalization at the age of 1 and 5
207 months , 1 episode of febrile seizures at the age of 2 months and 1 episode of extreme vomiting
208 at the age of 5 months. No congenital disorders were detected among the infants in the study.

209 Laboratory results

210 Table 3 provides an overview of the Geometric Mean Concentrations (GMCs) of IgG antibodies
211 to Tdap vaccine antigens in the sera from all mothers and infants.

212 At baseline, no significant differences were found between both groups for any measured
213 antibody. Protective antibody concentrations for tetanus and diphtheria were measured at all
214 other time points in mothers and infants. Women in the vaccine group had significantly higher
215 GMCs to all antigens at delivery compared with women from the control group, except for

tetanus (p=0.064). Significantly higher antibody concentrations were found in the cord blood of the vaccine group compared with the control group for all antigens, except again for tetanus (p=0.888).

Despite a significant decrease in antibody titers between birth and the age of 8 weeks, right before the administration of the first infant vaccine dose, the GMCs to all antigens were still significantly higher in infants from vaccinated mothers compared with infants from unvaccinated mothers (*Table 3*) at the age of 8 weeks.

At 1 month after the third hexavalent vaccine dose, GMCs to PT (p<0.001) (*Figure 2*) and DT (p=0.002) were significantly lower in the vaccine group compared with the control group. However, antibody GMCs for both antigens had risen from week 8 to month 5 (*Figure 1*). For Prn (p=0.220), TT (p=0.560) and FHA (p=0.198), non-significant differences in GMCs were found in the vaccine group compared to the control group. For these three antigens a decay in antibody titer from week 8 to month 5 is notified in the vaccine group (*Figure 1*).

Figure 1 demonstrates the log distribution of the prevaccination and postvaccination (after 3 doses) IgG titers for all vaccine antigens in both the vaccine and control group.There is a significant difference in the distribution of antibodies for all prevaccination titers in the vaccine group versus the control group, in favor of the vaccine group. The postvaccination titers differ significantly between both groups for PT and DT. *Figure 2* shows the individual data for PT antibodies only, but expressed as the individual correlation of pre-vaccination and post-vaccination IgG titers for each infant in both groups.

Transplacental transport rate

No significant difference was observed for the transplacental transport rate (Fetal/Maternal titer) for all three pertussis antibodies between both groups. For TT, a significant lower transplacental transport rate was found in the vaccine group, whereas for DT, a significantly higher transplacental transport rate was found in the vaccine group (*Table 4).*

Results from the regression analysis

We report only the significant influences of all variables included in the random forest analysis. At baseline, a higher parity had a positive effect on the anti-FHA antibody concentration in the control group (p=0.03), and older women had higher anti-Prn GMCs in the control group (p=0.017). A negative influence of the receipt of a tetanus-only vaccine within the past 10 years was observed on the anti-FHA antibody concentration (p=0.01) and the anti-Prn antibody concentration (p=0.04) at baseline in the control group.

At delivery, a higher parity had a positive effect on the anti-PT antibody concentration in the women in the vaccine group (p=0.01). A higher gestational age at delivery negatively influenced the anti-PT antibody concentration at delivery in the control group (p=0.01) and the anti-FHA antibody concentration in the vaccine group (p=0.004).

The gestational age at vaccination did not demonstrate an influence on the titer of antibodies in the cord. At both time points in the infants (week 8 and month 5), no significant influence on the antibody concentrations was encountered by any of the variables studied.

Discussion

This study is the first to investigate the effect of the administration of Boostrix® in pregnant women on transmitted maternal antibodies and to assess the immune responses of infants administered acellular pertussis-containing vaccines (Infanrix hexa®) according to a schedule of 8, 12 and 16 weeks of age. The presented data show that vaccinating during pregnancy closes the susceptibility gap for pertussis infection.

Safety data have been reported from far larger studies [16, 26], showing that pertussis vaccination during pregnancy is safe and well tolerated. The AE reported within this study (73.7% showed mild to moderate injection site pain and swelling) do not differ from the expected side effects described in the Boostrix® summary of product characteristics (SmPC: 23.7% - 80.6%) [27]. Because we did not use a placebo in the control group, it is not possible to make a comparison of the rate of adverse events between both groups. SAE were encountered in this study. However, the number of reported serious adverse events was small and did not differ from what is expected within the general population [28]. The safety data in the offspring did not demonstrate an unexpected risk pattern; no congenital disorders were detected.

Transplacental transport was effective in both groups for all vaccine antigens. Inter-pathogen specific IgG differences in the effectiveness of this transport have been described before [29], despite a common mechanism for transplacental IgG transport involving the FcRn receptor. Healy et al. reported a lower transplacental transport rate for PT compared with the rate reported in this study [30]. Conversely, for FHA and Prn, similar transport rates were found in both studies. In a randomized controlled trial, Muñoz et al [16] also reported an effective

transplacental transport for PT antibodies, similar to what we report in this study, but less for FHA, Prn, DT and TT. However, a comparison of serological results from various studies should be interpreted with caution because different laboratory techniques are utilized.

At baseline, all IgG GMCs were comparable in both groups. There was an adequate maternal immune response to all vaccine antigens except for tetanus, yet all women were already protected for tetanus at baseline. We showed in another paper [31] that humoral responses to pertussis vaccines in pregnancy are as robust as in non-pregnant women. The RCT performed by Muñoz et al [16] describes equally high antibody titers post-vaccination as during pregnancy. Again, immune responses in the latter study were measured with other laboratory techniques and the women were vaccinated with another vaccine brand (Adacel®); therefore, titers cannot easily be compared.

At delivery and in cord samples, IgG GMC were still significantly higher in the vaccine group compared with their baseline values for all vaccine antigens. These increased titers persisted until the age of 8 weeks, before the start of the primary vaccination schedule, suggesting a closure of the susceptibility gap of in newborns. A decay of maternal antibodies during the first weeks of life has been described before [16, 20], and although there is no known correlate of protection for pertussis, high concentrations of PT, FHA and Prn IgG are associated with protection against (severe) disease [32, 33]. A decay of maternal antibodies during the first weeks of life has been described before [16, 20]. Yet, at week 8, immediately preceding the vaccination, infants of vaccinated women still had significantly higher antibody titers compared with the control group.

Naturally acquired maternal pertussis antibodies have been shown to interfere with humoral responses to wP, yet not to aP vaccines [7, 34-39]. Nevertheless, the recent study by Muñoz et al showed a trend of blunting by maternal antibodies for FHA ($p<0.01$) after a 3-dose priming schedule [16]; however, this blunting effect disappeared with the booster dose offered during the second year of life, whereas the clinical significance of the interference has not been demonstrated. This result confirmed the finding by Hardy- Fairbanks et al [15]. In the study presented here, the blunting of the infant immune response is also suggested in infants from mothers in the vaccine group for anti-PT antibodies ($p<0.001$). The differences between our study and the Muñoz study could be due to different brands of vaccine used during pregnancy and to other confounders in both populations (e.g., different epidemiological background for pertussis, different vaccination histories, etc.). Jones et al measured the effect of high levels of maternal antibodies on the humoral immune responses to vaccines in infancy in general and found the inhibition of immune responses to tetanus and pneumococcus [40]. The present study does not confirm this blunting effect for tetanus, and pneumococcal antibody titers have not (yet) been analyzed. Mouse models seem to indicate however, that maternal antibodies, and blunting, do not solely have a negative effect on infant immune responses but might enhance the B cell maturation in infants [41]. In a study conducted in parallel in Vietnam, we describe less blunting effect by maternal antibodies. A possible explanation could be that we used different brands of acellular pertussis vaccines in mothers and infants in Vietnam (cross reference to manuscript JVAC-D-15-01293), resulting in different antibodies. This difference in interference was already shown in a mouse model experiment (personal communication Camille Locht, Institut Pasteur de Lille).

Abu Raya et al [18] also used Boostrix®vaccine during pregnancy, as in the present study, whereas Muñoz et al [16] and Hardy Fairbanks et al [15] used Adacel. A comparison of antibody titers induced by vaccines of distinct manufacturers has never been analyzed in a single pregnant population.

A random forest regression analysis revealed no consistent influence of any factor on the entire study population, except for vaccination during pregnancy. Only isolated significant influences of some variables on one specific time point in one specific group were described, never indicating any plausible relationship. Unlike Abu Raya et al [18], we could not confirm the influence of gestational age at vaccination on the titer of antibodies encountered in the cord. Our study was not powered for the analysis of this specific influence; considerably larger cohorts are needed to show this effect.

Limitations of the study

Strict randomization was not possible, as explained in the methods section. No significant differences in demographic characteristics were observed between the vaccine and control group, which suggested that the groups of pregnant women were comparable.

Another potential limitation of the study is that the results are unlikely generalizable to countries with different epidemiological profiles as well as other vaccine compositions and vaccination schedules, as the study was only performed in 1 province (Antwerp) in Belgium.

Conducting clinical trials in pregnant women and their offspring is difficult. Recruitment is time consuming and labor intensive. Moreover, it is a challenge to retain both mothers and infants

throughout the entire study period [42]. In our study, there were limited amounts of missing data (12.4%). Therefore, we performed a complete case analysis which assumes that the missingness process was unrelated to the observed and unobserved titer values. We were confronted with a large drop-out rate, especially in the control group, which resulted in wider confidence intervals of the results.

And lastly, despite validation of the laboratory results, comparison with other studies and laboratories remains a major challenge, as in many other trials.

Conclusion

The pertussis vaccination has been recommended for every pregnant woman during each pregnancy by the Superior Health Council in Belgium since August 2013 and many other countries in Western Europe and North America; the results of this study support these recommendations and provide additional scientific evidence to continue this vaccination strategy. The susceptibility gap for pertussis in the youngest age group, before immunization starts, was closed. Blunting was found for the anti-PT antibody immune response in infants of vaccinated women; however, follow-up of the children until after the booster dose at 15 months of age will shed further light on whether this blunting will persist.

Tables

		Vaccine group	Control group
N (women)		57	42
Mean age at delivery in years (SD)		30.7 (4.0)	32.3 (3.8)
Level of education, no. (%)			
	Unknown	1 (1.8)	0
	Secondary school	13 (22.8)	1 (2.4)
	Bachelor	18 (31.6)	23 (54.8)
	Master	25 (43.9)	18 (42.9)
Race mother, no. (%)			
	Caucasian	55 (96.5)	41 (97.6)
	Other	2 (3.5)	1 (2.4)
Mean gestational age at delivery in weeks (SD)		39.7 (1.4)	39.7 (1.0)
Primiparity, no. (%)		43 (75.4)	28 (66.7)
Mean gestational age at vaccination in weeks (SD)		28.6 (2.8)	NA
Mean interval between vaccination and delivery in days (SD)		77.1 (17.5)	NA
Tetanus dose <10 years ago, no. (%)		26 (45.6)	19 (45.2)
Exposure pertussis disease <10 years ago, no. (%)		0	1 (2.4)
Twin pregnancies, no. (%)		2 (3.5)	0
Mode of delivery, no. (%)			
	Vaginal	46 (80.7)	35 (83.3)
	Cesarean	11 (19.3)	7 (16.7)
Induction of labor, no. (%)		19 (33.3)	8 (19.0)
Epidural anesthesia, no. (%)		40 (70.2)	24 (57.1)
N (included infants)		55	26
Infant gender, no. (%)	Male	30 (50.8)	17 (40.5)
	Female	29 (49.2)	25 (59.5)
Mean weight at birth in gram (SD)		3351.7 (485.2)	3404.8 (479.4)
Mean length at birth in centimeters (SD)		50.3 (2.6)	49.5 (2.7)
Mean weight week 8 in gram (SD)		5165.6 (584.2)	5058.2 (492.3)
Mean length week 8 in centimeters (SD)		57.3 (2.2)	57.2 (1.9)
Mean weight month 5 in gram (SD)		7376.5 (940.9)	7345.9 (707.3)
Mean length month 5 in centimeters (SD)		66.2 (2.5)	66.6 (1.7)
Mean age at vaccine dose 1 in days (SD)		63.0 (7.4)	67.5 (7.4)
Mean age at vaccine dose 2 in days (SD)		95.9 (11.2)	101.5 (12.6)
Mean age at vaccine dose 3 in days (SD)		131.2 (15.1)	135.6 (16.6)
Mean age at blood sample before primary vaccination in days (SD)		55.9 (3.8)	55.2 (6.9)
Mean age at blood sample 1 month after primary vaccination in days (SD)		162.3 (15.1)	166.0 (16.5)
Mean interval between vaccine dose 3—blood sample month 5 in days (SD)		31.7 (3.0)	30.9 (2.8)

Table 1: Demographic and clinical characteristics of all study participants

	Vaccine group (*N* = 57)	Control group (*N* = 41)
Preterm preeclampsia (number/proportion)	1 (1.75%)	0 (0%)
Term preeclampsia (number/proportion)	3 (5.26%)	1 (2.44%)
Premature contractions	4 (7.02%)	0 (0%)
Hypertension	2 (3.50%)	1 (2.44%)
Oligohydramnion	1 (1.75%)	0 (0%)
Placenta praevia	0 (0%)	1 (2.44%)
Total number of serious adverse events	11	3

Table 2: Overview of the reported serious adverse events within the study.

GMC (95%CI)	Women						Infants					
	Baseline		1 Month after vaccination		At delivery		Cord		Before primary vaccination		1 Month after primary vaccination	
	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control
N	57 (54 for anti-PT)	31	57	0	57 (56 for anti-PT and anti-FHA)	41	58 (57 for anti-PRN)	41	51	26	49	21
Tetanus toxoid, IU/ml	1.5 (1.3–1.7)	1 .7 (1.4–2.1)	3 .6 (3.5–3.8)	NA	1.9 (1.6–2.3)	1.5 (1.2–1.7)	2.4 (2.3–2.5)	2.4 (1.9–2.9)	1.9 (1.8–2)	0.8 (0.7–1)	1.7 (1.7–1.8)	1.9 (1.7–2.1)
p-Value	0.212		NA		0.064		0.888		<0.001		0.560	
Diphtheria toxoid, IU/ml	0.3 (0.2–0.4)	0.3 (0.2–0.5)	1.4 (1.3–1.7)	NA	1.2 (1–1.5)	0.3 (0.2–0.4)	1.7 (1.5–2.1)	0.3 (0.2–0.5)	0.9 (0.7–1)	0.12 (0.1–0.17)	2.1 (1.9–2.2)	2.6 (2.4–2.9)
p-Value	0.749		NA		<0.001		<0.001		<0.001		0.002	
Pertussis toxin, IU/ml	4.5 (3.2–6.4)	7.5 (5–11)	48 (39–59)	NA	31.4 (26–38)	6.4 (4.3–9.6)	100.7 (82–123)	12.4 (8–19)	15.5 (12.1–20)	1.1 (0.7–1.6)	29 (25–35)	54 (42–69)
p-Value	0.078		NA		<0.001		<0.001		<0.001		<0.001	
Filamentous Heammaglutinin, IU/ml	21 (17–26)	17.6 (13–24)	211 (170–263)	NA	107 (91–126)	21.4 (16.6–27.5)	140 (109–180)	27.5 (21.5–35)	121 (100–145)	23 (19–27)	65 (56–75)	54 (41–70)
p-Value	0.409		NA		<0.001		<0.001		<0.001		0.198	
Pertactin, IU/ml	24 (18–31)	16.9 (11.6–24.6)	622 (511–756)	NA	602 (485.5–747)	18 (13–24)	697 (573–848)	21 (15.5–28)	253 (183–351)	17 (14.5–21)	68 (56–84)	87 (62–121)
p-Value	0.147		NA		<0.001		<0.001		<0.001		0.220	

366

367 Table 3: Geometric Mean Concentrations with 95% confidence interval (CI) for antibodies to
368 tetanus, diphtheria, pertussis toxin, filamentous haemaglutinin, pertactin in both groups of
369 women and infants

	Cord/maternal titer (SD)		
	Vaccine	Control	*p*-Value
Tetanus toxoid	1.17 (0.35)	1.65 (0.42)	<0.001
Diphtheria toxoid	1.42 (0.37)	1.20 (0.40)	0.007
Pertussis toxin	3.47 (1.40)	2.90 (3.52)	0.269
Filamentous haemagglutinin	1.81 (1.99)	1.35 (0.40)	0.096
Pertactin	1.24 (0.38)	1.33 (0.58)	0.322

370

371 Table 4: The rate of the cord titer/ maternal titer with standard deviation (SD) for tetanus,
372 diphtheria, pertussis toxin, filamentous haemaglutinin and pertactin in both study groups

373

374

Figures

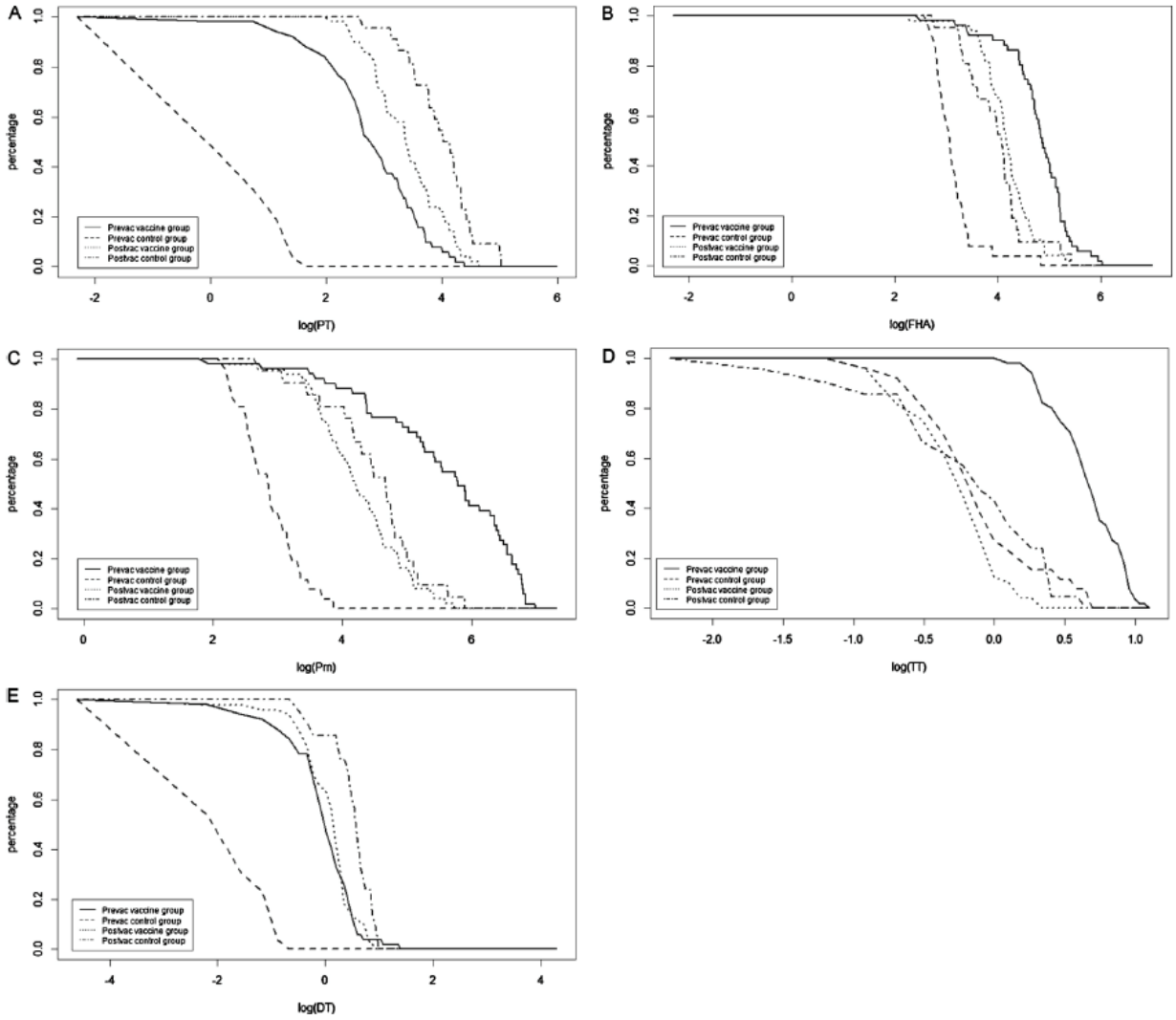
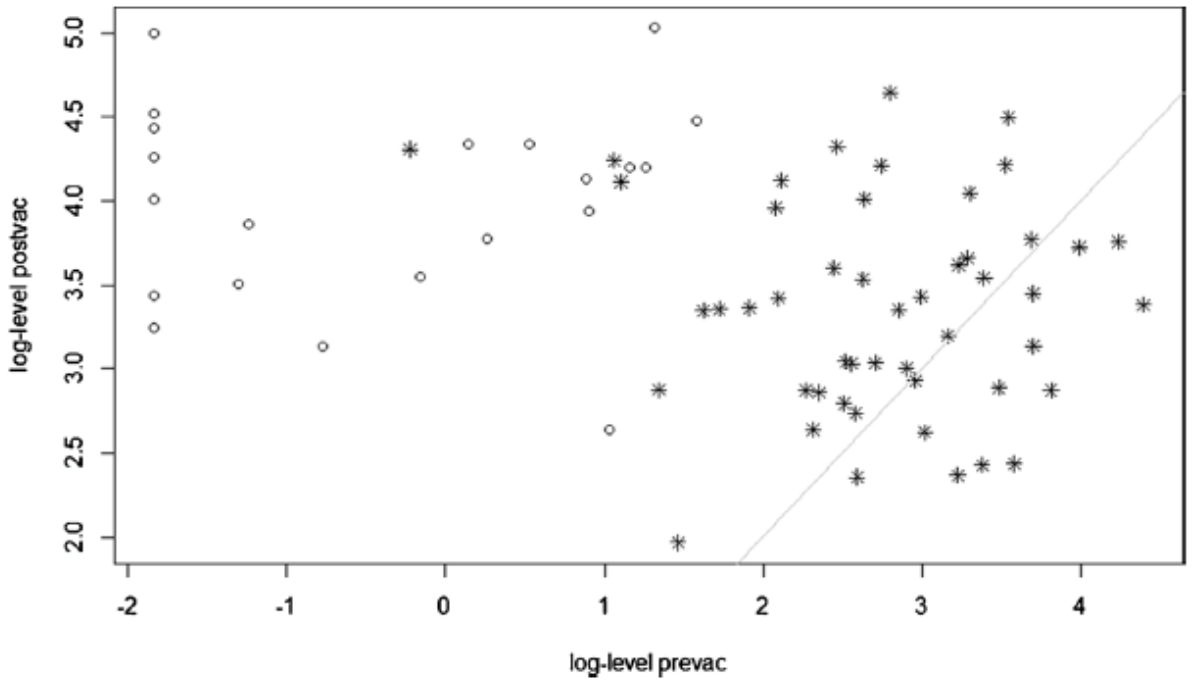


Figure 1: Comparison of both groups of infants before and after the priming vaccination regarding the antigen specific (log) antibody levels. 1A: Anti-PT antibodies. 1B: Anti-FHA antibodies. 1C: Anti-Prn antibodies. 1D: Anti-TT antibodies. 1E: Anti-DT antibodies



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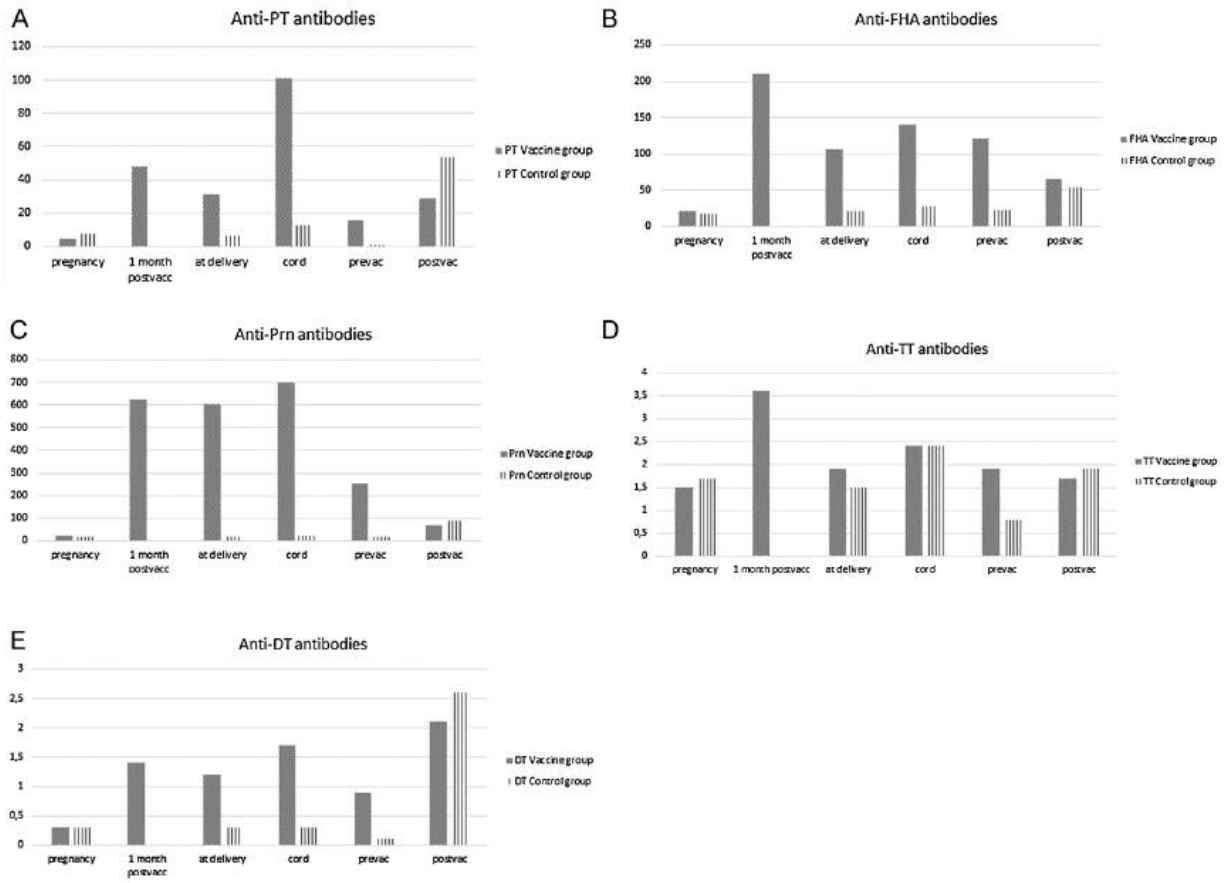
381 Figure 2: The individual correlation of the anti-PT antibody titers pre- and post-primary

382 vaccination in infants in the control group (dots) and the vaccine group (stars).



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384 Figure 3: Geometric Mean Concentrations for antibodies to tetanus (TT), diphtheria (DT),

385 pertussis toxin (PT), Filamentous Haemmaglutinin (FHA) and Pertactin (Prn) in both groups of

386 women and infants at all time points.

387 **Legends**

388 Table 1: Demographic and clinical characteristics of all study participants

389 Table 2: Overview of the reported serious adverse events within the study.

Table 3: Geometric Mean Concentrations with 95% confidence interval (CI) for antibodies to tetanus, diphtheria, pertussis toxin, filamentous haemaglutinin, pertactin in both groups of women and infants

Figure 1: Comparison of both groups of infants before and after the priming vaccination regarding the antigen specific (log) antibody levels. 1A: Anti-PT antibodies. 1B: Anti-FHA antibodies. 1C: Anti-Prn antibodies. 1D: Anti-TT antibodies. 1E: Anti-DT antibodies

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Table 4: The rate of the cord titer/ maternal titer with standard deviation (SD) for tetanus, diphtheria, pertussis toxin, filamentous haemaglutinin and pertactin in both study groups

Figure 3: Geometric Mean Concentrations for antibodies to tetanus (TT), diphtheria (DT), pertussis toxin (PT), Filamentous Haemmaglutinin (FHA) and Pertactin (Prn) in both groups of women and infants at all time points.

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References

[1] WHO. Immunization, Vaccines and Biologicals: Pertussis. 2014. Available from: http://www.who.int/wer/2015/wer9035.pdf?ua=1. Accessed on 18/10/2015.

[2] Pebody RG, Gay NJ, Giammanco A, Baron S, Schellekens J, Tischer A, et al. The seroepidemiology of Bordetella pertussis infection in Western Europe. Epidemiology and infection 2005 Feb;133(1):159-71.

[3] Crowcroft NS, Pebody RG. Recent developments in pertussis. Lancet 2006 Jun 10;367(9526):1926-36.

[4] Crowcroft NS, Stein C, Duclos P, Birmingham M. How best to estimate the global burden of pertussis? The Lancet infectious diseases 2003 Jul;3(7):413-8.

[5] Hoge Gezondheidsraad België. Pertussis vaccination. 2013. Available from: https://www.zorg-en-gezondheid.be/uploadedFiles/NLsite_v2/Ziekten/Vaccinaties/Informatie_voor_vaccinatoren/vaccinatie_fiche_volw_kinkhoest_20130830.pdf. Accessed on: 18/10/2015.

[6] Braeye T, De Schrijver K, Pierard D, Huygen K. Infectieziekten bij kinderen, die voorkomen kunnen worden door vaccinatie. Kinkhoest. Jaarrapport 2013. 2013. Available from: https://www.wiv-isp.be/SiteCollectionDocuments/Infectieziekten%20bij%20kinderen,%20die%20voorkomen%20kunnen%20worden%20door%20vaccinatie.%20Jaarrapport%202013.pdf. Accessed on: 18/10/2015.

[7] Van Savage J, Decker MD, Edwards KM, Sell SH, Karzon DT. Natural history of pertussis antibody in the infant and effect on vaccine response. The Journal of infectious diseases 1990 Mar;161(3):487-92.

[8] Edwards KM. Pertussis: an important target for maternal immunization. Vaccine 2003 Jul 28;21(24):3483-6.

[9] Malek A, Sager R, Schneider H. Maternal-Fetal Transport of Immunoglobulin-G and Its Subclasses During the 3Rd Trimester of Human-Pregnancy. American Journal of Reproductive Immunology 1994;32(1):8-14.

[10] Clark TA. Changing pertussis epidemiology: everything old is new again. The Journal of infectious diseases 2014 Apr 1;209(7):978-81.

[11] De Schutter S, Maertens K, Baerts L, De Meester I, Van Damme P, Leuridan E. Quantification of Vaccine-induced Antipertussis Toxin Secretory IgA Antibodies in Breast Milk: Comparison of Different Vaccination Strategies in Women. The Pediatric infectious disease journal 2015 Jun;34(6):e149-52.

[12] Lindsey B, Kampmann B, Jones C. Maternal immunization as a strategy to decrease susceptibility to infection in newborn infants. Current opinion in infectious diseases 2013 Jun;26(3):248-53.

[13] Leuridan E, Hens N, Hutse V, Aerts M, Van Damme P. Kinetics of maternal antibodies against rubella and varicella in infants. Vaccine 2011 Jun 15;29(11):2222-6.

[14] Gall SA, Myers J, Pichichero M. Maternal immunization with tetanus-diphtheria-pertussis vaccine: effect on maternal and neonatal serum antibody levels. American journal of obstetrics and gynecology 2011 Apr;204(4):334 e1-5.

[15] 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. The Pediatric infectious disease journal 2013 Nov;32(11):1257-60.

[16] 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 2014 May 7;311(17):1760-9.

[17] Vilajeliu A, Gonce A, Lopez M, Costa J, Rocamora L, Rios J, et al. Combined tetanus-diphtheria and pertussis vaccine during pregnancy: transfer of maternal pertussis antibodies to the newborn. Vaccine 2015 Feb 18;33(8):1056-62.

[18] 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 2014 Oct 7;32(44):5787-93.
[19] Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol 2010 Jul;17(7):1055-65.
[20] Leuridan E, Hens N, Peeters N, de Witte L, Van der Meeren O, Van Damme P. Effect of a Prepregnancy Pertussis Booster Dose on Maternal Antibody Titers in Young Infants. The Pediatric infectious disease journal 2011 Jan 4 30(7):608-10.
[21] Neter J, Kutner M, Nachtsheim C, Wasserman W. Applied Linear Statistical Models. (4th edition). Chicago: Irwin, 1996.
[22] Agresti A. Categorical data analysis. 2nd edition. New York: Wiley, 2002.
[23] Little R, Rubin D. Statistical Analysis with Missing Data.: Wiley, 1987.
[24] Breiman L. Random forests. Machine Learn 2001;45(1):5-32.
[25] Theeten H, Hens N, Vandermeulen C, Depoorter AM, Roelants M, Aerts M, et al. Infant vaccination coverage in 2005 and predictive factors for complete or valid vaccination in Flanders, Belgium: an EPI-survey. Vaccine 2007 Jun 21;25(26):4940-8.
[26] Donegan K, King B, Bryan P. Safety of pertussis vaccination in pregnant women in UK: observational study. BMJ Clinical research ed 2014;349:g4219.
[27] GlaxoSmithKline Inc. Product monograph Boostrix. 2013.
[28] Jacquemyn Y, Osmanovic F, Martens G. Preeclampsia and birthweight by gestational age in singleton pregnancies in Flanders, Belgium: a prospective study. Clinical and experimental obstetrics & gynecology 2006;33(2):96-8.
[29] de Voer RM, van der Klis FR, Nooitgedagt JE, Versteegh FG, van Huisseling JC, van Rooijen DM, et al. Seroprevalence and placental transportation of maternal antibodies specific for Neisseria meningitidis serogroup C, Haemophilus influenzae type B, diphtheria, tetanus, and pertussis. Clin Infect Dis 2009 Jul 1;49(1):58-64.
[30] Healy CM, Rench MA, Baker CJ. Importance of timing of maternal combined tetanus, diphtheria, and acellular pertussis (Tdap) immunization and protection of young infants. Clin Infect Dis 2013 Feb;56(4):539-44.
[31] Huygen K, Cabore RN, Maertens K, Van Damme P, Leuridan E. Humoral and cell mediated immune responses to a pertussis containing vaccine in pregnant and nonpregnant women. Vaccine 2015;33:4117-23.
[32] Mooi FR, de Greeff SC. The case for maternal vaccination against pertussis. The Lancet infectious diseases 2007 Sep;7(9):614-24.
[33] Heininger U, Riffelmann M, Bar G, Rudin C, von Konig CH. The protective role of maternally derived antibodies against Bordetella pertussis in young infants. The Pediatric infectious disease journal 2013 Jun;32(6):695-8.
[34] Englund JA, Anderson EL, Reed GF, Decker MD, Edwards KM, Pichichero ME, et al. The effect of maternal antibody on the serologic response and the incidence of adverse reactions after primary immunization with acellular and whole-cell pertussis vaccines combined with diphtheria and tetanus toxoids. Pediatrics 1995 Sep;96(3 Pt 2):580-4.
[35] Sako W TW, Witt DB, et al. Early immunization against pertussis with alum precipitated vaccine. JAMA : the journal of the American Medical Association 1945;127:379-83.
[36] Glezen WP. Effect of maternal antibodies on the infant immune response 3. Vaccine 2003;21(24):3389-92.
[37] Provenzano RW WL, Sullivan CL. . Immunization and antibody response in the newborn infant. The New England journal of medicine 1965;273:959-65.

[38] Saffar MJ, Ajami A, Khalilian AR, Qaheri A, Saffar H. Pertussis seroimmunity among mother-infant pairs and infant immune response to pertussis vaccination. Indian pediatrics 2007 Dec;44(12):916-8.
[39] Van Rie A, Wendelboe AM, Englund JA. Role of maternal pertussis antibodies in infants. The Pediatric infectious disease journal 2005 May;24(5 Suppl):S62-5.
[40] Jones C, Pollock L, Barnett SM, Battersby A, Kampmann B. The relationship between concentration of specific antibody at birth and subsequent response to primary immunization. Vaccine 2014 Feb 12;32(8):996-1002.
[41] Niewiesk S. Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. Frontiers in immunology 2014;5:446.
[42] Frew PM, Saint-Victor DS, Isaacs MB, Kim S, Swamy GK, Sheffield JS, et al. Recruitment and retention of pregnant women into clinical research trials: an overview of challenges, facilitators, and best practices. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2014 Dec 15;59 Suppl 7:S400-7.

Conflict of interest statement

Authors do not have a commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding);