Optimising the timing of whooping cough immunisation in mums (OpTIMUM) through investigating pertussis vaccination in pregnancy: an open-label, equivalence, randomised controlled trial

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Summary

Background Pertussis vaccination in pregnancy is recommended in many countries to provide protection to young infants. The best timing for this vaccination is uncertain. In the UK, vaccination is recommended between 16 weeks and 32 weeks of gestation. In this trial we aimed to investigate the equivalence of three time periods for pertussis vaccination in pregnancy.

Methods In this open-label, equivalence, randomised controlled trial to investigate equivalence of different time windows for pertussis vaccination in pregnancy, participants were randomly assigned (1:1:1 ratio) to receive a pertussis-containing vaccine (Boostrix-inactivated poliovirus vaccine) in one of three gestational age groups, comprising group 1 (≤23 weeks + 6 days), group 2 (24–27 weeks + 6 days), and group 3 (28–31 weeks + 6 days) using a computer-generated randomisation list. The primary outcome was concentration of pertussis-specific antibodies in the infant born at term at birth. Maternal blood sampling was done before and 2 weeks after vaccination and at delivery, together with a cord sample, and an infant sample was collected at least 4 weeks after primary vaccination. Reactogenicity was assessed for 7 days after vaccination. This trial was registered with ClinicalTrials.gov (NCT03908164).

Findings Between May 7, 2019, and Feb 13, 2020, of 1010 women assessed for eligibility, 364 women were recruited and 351 received the intervention (120 in group 1, 119 in group 2, and 112 in group 3). Equivalence of time periods was demonstrated for anti-pertussis toxin and anti-pertactin IgG concentrations. The cord blood geometric mean concentrations of anti-filamentous haemagglutinin IgG were higher with increasing gestational age at vaccination, such that for infants in group 1 (≤23 weeks + 6 days), equivalence to group 3 (28–31 weeks + 6 days) was not shown. Reported rates of fever were similar between study groups.

Interpretation Pertussis vaccination at three different time intervals in pregnancy resulted in equivalent concentrations of IgG antibodies in infants against two of the three pertussis antigens assessed. Overall, these findings support recommendations to vaccinate any time between 16 weeks and 32 weeks of gestation.

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Introduction

Pertussis is a highly infectious respiratory disease caused by the bacterium *Bordetella pertussis*. The clinical presentation of pertussis is variable, but in young infants disease can be severe, particularly in infants not yet vaccinated. Routine pertussis vaccination was introduced into the UK childhood immunisation schedule in 1957, using a whole-cell vaccine, and subsequently the incidence of pertussis disease decreased substantially.[1] In many countries, including those with good vaccine coverage, there was an increase in cases from around 2005, associated with an increase in cases of hospitalisation and death in young infants.[2,3] In response, pertussis vaccination in pregnancy has been introduced in many countries. Pertussis vaccination in pregnancy increases anti-pertussis IgG in women, which leads to increased transplacental transfer of IgG. This increase results in a higher anti-pertussis IgG concentration in infants, providing protection until they are vaccinated. Pertussis vaccination in pregnancy is safe for both mother and infant,[4] and effective in preventing pertussis disease, hospitalisation, and death in infants.[4–7] However, the best time to offer vaccination in pregnancy to provide optimal protection for infants is debated.

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Research in context

Evidence before this study

We did a systematic review of studies investigating the timing of pertussis vaccination in pregnancy that were published between Jan 1, 2000, and Dec 31, 2019. We searched Embase and MEDLINE on Aug 11, 2022, using the search string "whooping cough" OR "pertussis" AND "maternal vaccination" OR "antenatal vaccination" OR "vaccination in pregnancy" OR "vaccination during pregnancy" AND "timing" OR "time interval" OR "gestational age" OR "gestation" and MESH terms "*Bordetella pertussis*" and "maternal vaccination", restricted to English-language papers only. We found no randomised controlled trials and six observational studies. In terms of antibody concentrations, two trials showed superiority of early third-trimester vaccination compared with vaccination later in the third trimester, whereas one trial showed no difference between these time periods and one further study showed that second trimester vaccination was superior to vaccination in the third trimester. Two studies investigated antibody avidity; one showed increased avidity of antibodies with increasing gestation at vaccination, whereas the other reported reduced avidity of antibodies with increasing gestation.

Added value of this study

We report the results of the first randomised controlled trial to investigate the effect of timing of pertussis vaccination in pregnancy on infant antibody concentrations. We found that for the three time intervals considered, antibody concentrations to two of the three pertussis antigens were equivalent (pertussis toxin and pertactin), whereas for one antigen (filamentous haemagglutinin), vaccination at 28–32 weeks of gestation resulted in higher antibody concentrations than vaccination earlier than 24 weeks of gestation.

Implications of all the available evidence

A wider time interval for pertussis vaccination in pregnancy might allow for higher vaccine coverage and improved protection for infants born prematurely. Considered together with vaccine effectiveness data, the evidence from this trial supports vaccination of pregnant women at any time between 16 weeks and 32 weeks of gestation.

In the UK, the initial recommendation was to administer the vaccine at 28–32 weeks of gestation because of concerns that the rapid rate of decay of pertussis-specific IgG would result in less protection for the infant if vaccination was administered earlier.

Several observational studies have suggested that vaccination earlier in the third trimester is superior to vaccination later in the third trimester. An Israeli study reported higher anti-pertussis toxin and anti-filamentous haemagglutinin IgG concentrations in the cord blood of infants born to mothers vaccinated at 27–30 weeks of gestation than those born to mothers vaccinated at 31–36 weeks of gestation.[8] An Australian study found that anti-pertussis toxin IgG concentrations were significantly higher in the cord blood of neonates born to mothers vaccinated at 28–32 weeks of gestation than in those born to mothers vaccinated at 33–36 weeks of gestation, with higher IgG avidity.[9,10] Another prospective cohort study showed that vaccination at 27–31 weeks of gestation resulted in higher anti-pertussis toxin antibody concentrations at birth than vaccination at 32–36 weeks of gestation, with concentrations increasing from 27 weeks to 30 weeks of gestation.[11] Finally, an observational study from Switzerland in 2016 showed that anti-pertussis toxin and anti-filamentous haemagglutinin concentrations were higher in cord blood following vaccination in the second trimester than following vaccination in the third trimester.[12] Following this report, and mindful of the logistical benefits of offering vaccination in a wider time window, the UK extended the recommended window for vaccination to 16–32 weeks in 2016.

With regard to vaccine effectiveness, two previous studies have shown that vaccination in the third trimester is more effective than vaccination in the second trimester,[13,14] whereas another study showed no effect of timing of vaccination on protection.[15]

We did an open-label, equivalence, randomised controlled trial to investigate the effect of timing of vaccine administration in pregnancy on the immunogenicity of a combined diphtheria, tetanus, and acellular pertussis (TdaP) vaccine.

Methods

Study design and participants

We did an open-label, equivalence, randomised controlled trial to investigate equivalence of different time windows for pertussis vaccination in pregnancy. Full details of the methods have been published previously.[16] We recruited from six UK sites: St George's University Hospitals UK National Health Service (NHS) Foundation Trust, Kingston Hospital NHS Foundation Trust, University Hospital Southampton NHS Foundation Trust, Oxford University Hospitals NHS Foundation Trust, University Hospitals Bristol NHS Foundation Trust, and Manchester University NHS Foundation Trust.

Participants were approached by letter, or as they attended routine antenatal care. All participants were recruited before 23 weeks + 6 days of gestation. Women were eligible to participate if they were pregnant and had not received pertussis vaccination in the current pregnancy, if they were willing and able to take part in the study and provide informed consent, and if they had a routine anomaly ultrasound scan at 20 weeks with no

evidence of life-limiting congenital abnormalities. Women were excluded from participation if they were younger than 16 years, if they had confirmed or suspected pertussis infection in the previous 5 years (identified through directed questions at the screening visit), if they had a known immune deficiency or had received immunosuppressive medication within 6 months of screening, or if, in the opinion of the investigator, they were unlikely to complete follow-up.

We did the trial according to the Declaration of Helsinki and the guidelines of Good Clinical Practice. Approval was received from the NHS Health Research Authority and York and Humber Research Ethics Committee (19/YH/0050). Participants provided written informed consent at recruitment for their own participation and for the future participation of their infant, with verbal consent for the infant's ongoing participation being confirmed with at least one parent following delivery. There was no additional written consent taken for the participation of the infant following delivery. This trial was registered with ClinicalTrials.gov (NCT03908164) on April 9, 2019.

Randomisation and masking

A computerised block-randomisation list was produced by the study statistician and participants were randomly assigned on a 1:1:1 ratio to one of the three following timing groups: group 1 (≤23 weeks + 6 days), group 2 (24–27 weeks + 6 days) and group 3 (28–31 weeks + 6 days). Group allocations were placed inside opaque envelopes bearing the corresponding participant number by staff not involved in the trial. Each centre was provided with the necessary envelopes and on recruitment to the study each participant was allocated, in order of inclusion, the next available participant number. There was no masking in the clinic. Participants and research staff were aware of the group allocation; however, laboratory staff who did the testing were not.

Procedures

At the screening visit randomisation took place and the timing of the vaccination visit was assigned. At the vaccination visit a baseline blood sample was collected before the participants received Boostrix-inactivated poliovirus vaccine (GlaxoSmithKline; London, UK). Boostrix-IPV contains pertussis toxin (8 µg), filamentous haemagglutinin (8 µg), pertactin (2·5 µg), diphtheria toxoid (not less than two international units), tetanus toxoid (not less than 20 international units), and inactivated polio virus types 1–3 (type-1 40 D-antigen unit, type-2 8 D-antigen unit, and type-3 32 D-antigen unit). Following vaccination, participants received a diary card for the 7 days following vaccination. A blood sample was collected 2 weeks following vaccination, together with the completed diary card, and participants were asked about adverse events. Following delivery, a cord sample and maternal sample were collected. If a cord sample was not obtained, parents were asked for permission to collect a sample from the infant within the first week of life. All infants were vaccinated in primary care according to the UK national schedule which included diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated poliovirus, haemophilus influenzae type B vaccine (Infanrix hexa; GlaxoSmithKline) at 2 months, 3 months, and 4 months of age. An infant visit then took place 28–70 days following completion of the primary vaccination series, at which details of vaccination, history of respiratory illness, or contact with cases of pertussis were recorded and a blood sample was collected. Maternal adverse events, which occurred within 28 days of vaccine administration, and medically attended adverse events or serious adverse events occurring during study participation for mother or infant were recorded.

Outcomes

Our primary objective was to determine whether pertussis vaccination at three different time intervals in pregnancy results in equivalent concentrations of pertussis-specific IgG in the term infant at birth. Our secondary objectives were as follows: to determine whether pertussis vaccination at different time intervals in pregnancy resulted in equivalent concentrations of pertussis-specific antibodies in the preterm infant at birth; to investigate the incidence of fever and local reactions in women receiving the vaccine in pregnancy who had not within 5 years or within a previous pregnancy received a pertussis-containing vaccine compared with those who had; to describe the kinetics of the antibody response to pertussis vaccination during pregnancy; to describe the transplacental transfer ratio of antibody following administration of vaccine at different timepoints; to explore the effect of repeated vaccination on the antibody response in women who had within 5 years or within a previous pregnancy received a pertussis vaccination; and to evaluate the effect of timing of pertussis vaccination in pregnancy on antibody concentrations in infants following their primary immunisation schedule.

Outcome assessment

Blood samples received in serum-separating tubes at the UK Health Security Agency (UKHSA) Porton Down were centrifuged, and the resulting serum was stored at –80°C until testing. Serum samples were analysed at UKHSA Porton Down. All samples had IgG antibody against pertussis toxin, filamentous haemagglutinin, and pertactin, measured using in-house, validated, ELISA techniques.[17] These concentrations were measured relative to the first WHO International Pertussis Standard Serum (06/140, National Institute for Biological Standards and Control). There is no correlation between specific concentrations of antibody against pertussis antigens and protection against pertussis disease. However, Eberhardt and colleagues[12] described a method

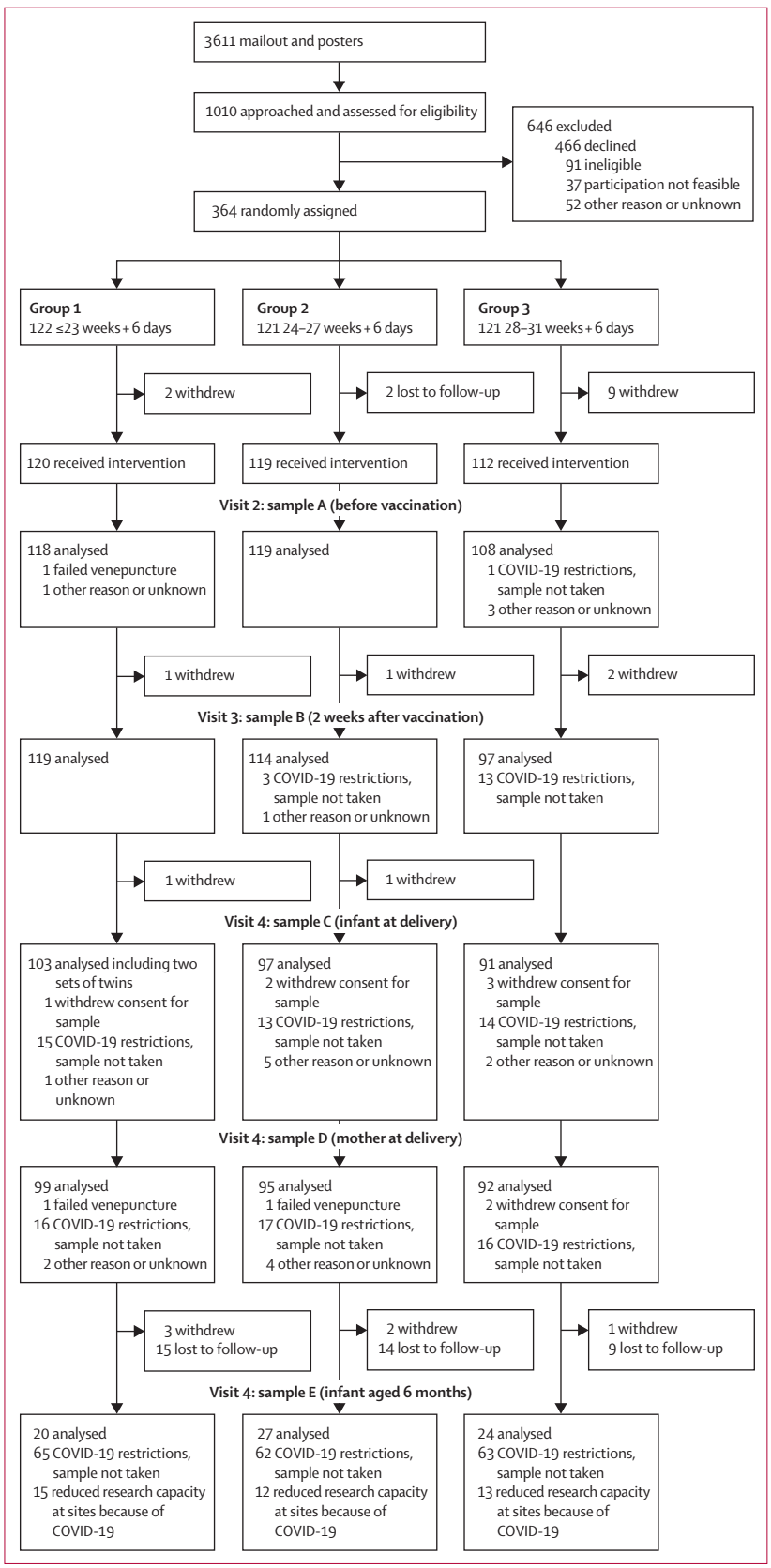

Figure 1: **CONSORT flow diagram for participants**

of defining infant seropositivity in which infants who were born with an anti-pertussis toxin concentration of more than 30 IU/mL were calculated to have antibody concentrations higher than 5 IU/mL until at least 3 months of age. As an additional exploratory analysis, we therefore compared the percentage of infants in each group that had anti-pertussis toxin concentrations higher than 30 IU/mL at birth, and these infants were considered to be seropositive.

Participants completed a diary card for 7 days following vaccination, which included recording their temperature and any local reactions once per day.

Statistical analysis

We calculated sample size using previous studies of cord blood, which showed the $\log_{10}$ SD to be about 0·50 for pertussis toxin, 0·40 for filamentous haemagglutinin, and 0·55 for pertactin. To assess equivalence within a 1·8-fold margin, and assuming the higher standard deviation of 0·55, we calculated that 100 women per group would be needed (two-sided 95% CI on the fold difference to assess equivalence, 80% power), which, allowing for a dropout rate of around 10% and a rate of prematurity of around 8%, would require recruitment of 354 women. Missing data were assumed to be missing at random and there was no imputation. The analysis was done per protocol. For reactogenicity, the analysis set included all women who received a dose of vaccine.

The primary outcome analysis investigated whether pertussis vaccination at different timepoints in pregnancy resulted in equivalent concentrations of pertussis-specific antibodies in the infant born at term. Antigen-specific geometric mean concentrations (GMCs) were calculated for each group with 95% CIs and equivalence assessed by calculating the geometric mean ratio compared with group 3 (later gestation) with equivalence shown if the 95% CI was contained within the equivalence margin (upper end 1·8 and lower end 0·55, equal to 1/1·8). Analyses of maternal antibody levels, transfer ratios, and antibody concentrations in infants following completion of their primary vaccination series assessed whether there were differences between groups 1 and 2 to group 3 (5% significance level) rather than equivalence. The geometric mean transfer ratio was the geometric mean of the ratio of infant-to-maternal antibody concentrations at birth using cord blood samples or, if these were not available, infant samples taken within 7 days of delivery. The effect of previous pertussis vaccinations in the mother was assessed by comparing maternal pertussis antigen-specific GMCs in women who had received none, one, or more than one recent pertussis-containing vaccine. Recent vaccination was defined as vaccination with a pertussis-containing vaccine in a previous pregnancy or within the past 5 years. Reactogenicity was estimated on the basis of the most severe level of reaction reported and presented as a percentage with 95% CI. An analysis was done of the

effect of previous vaccination on reactogenicity. A calculation of the predicted seropositivity of infants at 3 months was done as described in the statistical methods. Further details of the statistical analysis are included in the statistical analysis plan (appendix p 4). As the trial used a licensed vaccine already recommended in pregnancy, neither a data-monitoring committee nor interim analysis were required.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Participants were recruited from May 7, 2019, until Feb 13, 2020. Of 1010 women assessed for eligibility, 364 were randomly assigned, of whom 351 (120 in group 1, 119 in group 2, and 112 in group 3) received the intervention and were considered to be participants in the study. 330 women delivered at term, 15 women delivered preterm, and six women withdrew from the study before delivery (figure 1). The three groups were similar in their baseline characteristics (table 1).

The GMCs of specific IgGs of the three tested pertussis antigens are shown (figure 2; appendix p 1). For filamentous haemagglutinin, there were higher GMCs with increasing gestational age at vaccination, such that for the earliest gestational age (group 1, ≤23 weeks + 6 days) equivalence to group 3 (28–31 weeks + 6 days) was not achieved, and both groups 1 and 2 showed a significantly lower GMC than group 3 (group 3, 322·3 IU/mL, 95% CI 272·8–380·6, *vs* group 1, 189·1 IU/mL, 163·2–219·1; p≤0·001; ratio of group 1 to group 3, 0·59, 95% CI 0·48–0·72; group 3 *vs* group 2, 232·4 IU/mL, 202·2–267·2; p=0·003; ratio of group 2 to group 3, 0·72, 0·58–0·89). For pertussis toxin and pertactin, differences were smaller, and equivalence criteria were met (pertussis toxin: group 3, 67·3 IU/mL, 56·7–79·8, *vs* group 1, 51·2 IU/mL, 43·7–60·1; p=0·018; ratio of group 1 to group 3, 0·76, 0·61–0·95; group 3 *vs* group 2, 61·8 IU/mL, 53·1–72·0; p=0·47; ratio of group 2 to group 3, 0·92, 0·73–1·15; pertactin: group 3, 309·9 IU/mL, 229·3–418·8, *vs* group 1, 268·9 IU/mL, 205·6–351·8; p=0·49; ratio of group 1 to group 3, 0·87, 0·58–1·30; group 3 *vs* group 2, 271·4 IU/mL, 200·7–367·0; p=0·53; ratio of group 2 to group 3, 0·88, 0·58–1·32).

There was no significant difference in infants born at term reaching seropositivity between study groups (group 1, 74 [75·5%] of 98; group 2, 75 [80·7%] of 93; and group 3, 71 [83·5%] of 85; p=0·40).

GMCs of specific IgGs to pertussis toxin, pertactin, and filamentous haemagglutinin antigens for infants born preterm are shown (appendix pp 1–2). There were no significant differences for preterm infants between study groups in the GMCs of the three tested pertussis antigens; however, there were only four infants in each group.

	Group 1: ≤23 weeks + 6 weeks (n=119)	Group 2: 24–27 weeks + 6 weeks (n=119)	Group 3: 28–31 weeks + 6 weeks (n=113)
Maternal age in years, median (range)	33 (18–43)	33 (22–47)	33 (19–43)
Maternal White ethnicity	108 (91%)	105 (88%)	99 (88%)
Maternal body-mass index in kg/m², median (range)	24·1 (16·9–41·8)	24 (16·0–42·0)	24 (18·0–57·0)
Previous deliveries			
0	73 (61%)	76 (64%)	67 (59%)
1	39 (33%)	32 (27%)	34 (30%)
2	7 (6%)	8 (7%)	8 (7%)
3	0	0	3 (3%)
≥4	0	3 (3%)	1 (<1%)
Pertussis vaccines received in a previous pregnancy			
0	82 (69%)	87 (73%)	74 (66%)
1	33 (28%)	26 (22%)	38 (34%)
2	5 (4%)	6 (5%)	1 (<1%)
Gestational age at vaccination (all infants) in weeks, median (range)	21 + 3 (19 + 0 to 23 + 6)	25 + 3 (24 + 0 to 27 + 6)	28 + 5 (28 + 0 to 31 + 5)
Preterm deliveries	5 (4%)	5 (4%)	5 (4%)
Gestational age at birth (preterm infants) in weeks, median (range)	33 + 5 (24 + 3 to 36 + 0)	33 + 2 (31 + 4 to 36 + 6)	35 + 6 (34 + 4 to 36 + 1)

Table 1: **Baseline characteristics of participants**

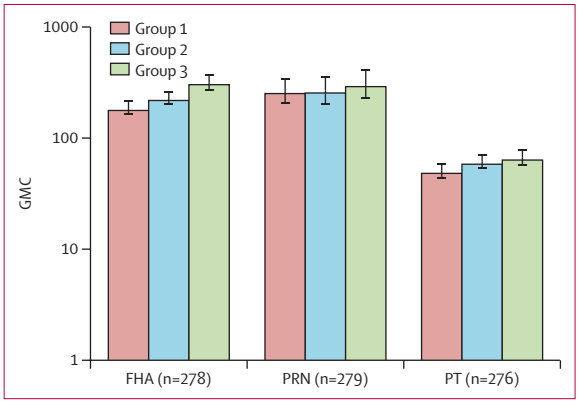

Figure 2: **GMC (IU/mL) and 95% CI of IgG against filamentous haemagglutinin, pertactin, and pertussis toxin in cord blood of infants born at term according to study group**
GMC=geometric mean concentration. FHA=filamentous haemagglutinin. PT=pertussis toxin. PRN=pertactin.

See **Online** for appendix

Transfer ratios for the three tested pertussis antigens are shown (appendix p 2). There were no differences in transfer ratios between study groups for either term or preterm infants (term infants: filamentous haemagglutinin, group 1, 1·88, 95% CI 1·77–2·00; group 2, 1·97, 1·83–2·11; group 3, 1·80, 1·68–1·93; group 1 *vs* group 3, p=0·34; group 2 *vs* group 3, p=0·065; pertactin, group 1, 1·84, 1·74–1·95; group 2, 1·91, 1·78–2·06; group 3, 1·74, 1·63–1·87; group 1 *vs* group 3, p=0·23; group 2 *vs* group 3, p=0·050; pertussis toxin, group 1, 1·84, 1·73–1·95; group 2, 1·87, 1·74–2·00; group 3, 1·71,

	Filamentous haemagglutinin			Pertactin			Pertussis toxin		
	GMC IU/mL (95% CI)	Ratio to group 3 (95% CI)	p value	GMC IU/mL (95% CI)	Ratio to group 3 (95% CI)	P value	GMC IU/mL (95% CI)	Ratio to group 3 (95% CI)	P value
Prevaccination									
Group 1: ≤23 weeks + 6 days (n=117)	26·5 (22·4–31·4)	0·89 (0·69–1·16)	0·39	19·9 (14·4–27·5)	0·94 (0·59–1·49)	0·78	10·4 (8·5–12·6)	1·08 (0·82–1·42)	0·59
Group 2: 24–27 weeks + 6 days (n=119)	27·2 (22·7–32·4)	0·91 (0·70–1·18)	0·50	16·4 (12·1–22·1)	0·77 (0·49–1·22)	0·27	10·7 (9·0–12·7)	1·11 (0·84–1·46)	0·46
Group 3: 28–31 weeks + 6 days (n=109)	29·8 (24·1–36·8)	..	..	21·2 (14·9–30·2)	..	..	9·6 (7·8–11·9)	..	..
2 weeks following vaccination									
Group 1: ≤23 weeks + 6 days (n=118)	156·0 (134·8–180·5)	0·70 (0·57–0·86)	0·001	208·7 (167·4–260·1)	0·87 (0·62–1·22)	0·42	46·7 (40·2–54·3)	0·85 (0·68–1·06)	0·15
Group 2: 24–27 weeks + 6 days (n=114)	183·2 (162·6–206·4)	0·82 (0·67–1·01)	0·058	195·7 (153·9–248·8)	0·81 (0·58–1·15)	0·24	55·0 (47·5–63·6)	1·00 (0·80–1·25)	1·0
Group 3: 28–31 weeks + 6 days (n=98)	222·6 (189·8–261·2)	..	..	240·5 (184·2–314·1)	..	..	55·0 (46·3–65·4)	..	..
At delivery									
Group 1: ≤23 weeks + 6 days (n=98)	100·9 (87·3–116·7)	0·57 (0·46–0·70)	<0·001	148·2 (114·0–192·6)	0·81 (0·54–1·20)	0·29	28·9 (24·6–34·0)	0·74 (0·59–0·93)	0·008
Group 2: 24–27 weeks + 6 days (n=95)	125·3 (109·1–143·9)	0·71 (0·58–0·87)	0·001	142·7 (107·7–189·1)	0·78 (0·52–1·16)	0·22	32·9 (28·1–38·6)	0·84 (0·68–1·06)	0·14
Group 3: 28–31 weeks + 6 days (n=93)	176·6 (150·4–207·4)	..	..	183·5 (134·6–250·0)	..	..	39·0 (33·4–45·5)	..	..

GMC=geometric mean concentration.

Table 2: **GMCs for filamentous haemagglutinin, pertactin, and pertussis toxin immunoglobulin G in maternal samples before and 2 weeks after vaccination and at delivery**

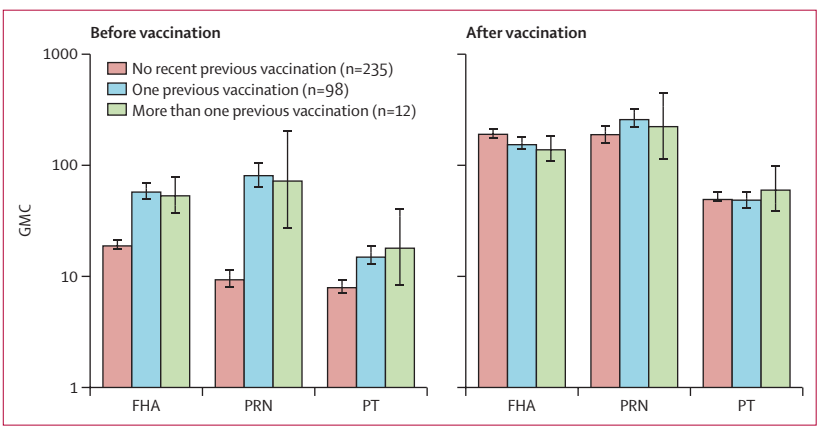

Figure 3: **GMC (IU/mL) and 95% CI for filamentous haemagglutinin, pertactin, and pertussis toxin immunoglobulin G according to recent vaccination status before and after receiving study vaccine**
FHA=filamentous haemagglutinin. GMC=geometric mean concentration. PT=pertussis toxin. PRN=pertactin.

1·58–1·85; group 1 *vs* group 3, p=0·14; group 2 *vs* group 3, p=0·075).

There were higher GMCs of specific IgG to pertussis toxin, pertactin, and filamentous haemagglutinin antigens in participants from group 3 at 2 weeks after receiving vaccination, but this increase was significant only for filamentous haemagglutinin when compared with group 1 and group 3 (group 3, 222·6 IU/mL, 95% CI 189·8–261·2, *vs* group 1 156·0 IU/mL, 134·8–180·5; p=0·001; table 2). At delivery, GMCs of filamentous haemagglutinin, pertactin, and pertussis toxin-specific IgG had waned versus 2 weeks after vaccination. This decrease was most evident in group 1 and group 2, in whom time since vaccination was greatest. The GMCs of specific IgG to the three tested pertussis antigens were higher in participants from group 3 for filamentous haemagglutinin than groups 1 and 2 (group 3, 176·6 IU/mL, 150·4–207·4, *vs* group 1, 100·9 IU/mL, 87·3–116·7 and group 3 *vs* group 2, 125·3 IU/mL, 109·1–143·9; p≤0·001 and p=0·001) and in participants from group 3 for pertussis toxin when compared with group 1 (group 3, 39·0 IU/mL, 33·4–45·5, *vs* group 1, 28·9 IU/mL, 24·6–34·0; p=0·008).

Recent TdaP vaccination was reported in 115 women; within the past 5 years and not related to pregnancy in six women, in a previous pregnancy in 107 women, and both within the past 5 years and in a previous pregnancy for two women. Before vaccination, there was a significant difference for all antigens between people who had not received recent vaccination and those who had received one or more recent vaccines. For filamentous haemagglutinin, the GMC for those receiving no recent vaccine was 19·5 IU/mL (95% CI 17·5–21·8) compared with 59·3 IU/mL (49·8–70·7) for one recent vaccine and 54·9 IU/mL (37·0–81·3) for more than one vaccine. For pertactin, the GMC was 9·6 IU/mL (7·9–11·6) for no recent vaccination, 82·7 IU/mL (63·2–108·4) for one recent vaccine, and 74·0 IU/mL (27·0–203·0) for more than one vaccine. For pertussis toxin, the GMC was 8·3 IU/mL (7·3–9·5) for

no recent vaccination, 15·7 IU/mL (13·2–18·7) for one recent vaccination, and 18·6 IU/mL (8·5–40·5) for more than one vaccine (figure 3; appendix p 2).

However, having received a recent TdaP vaccine resulted in no significant difference in GMCs following a subsequent dose of vaccine in pregnancy (appendix p 2).

There were no differences seen between any of the study groups in GMCs in infants after completion of their primary vaccination series (appendix p 3).

Reported local reactions or fever were similar between the three study groups (table 3).

When all recent diphtheria, tetanus, and polio vaccinations were included (in a previous pregnancy or in the past 5 years), only local tenderness following vaccination was reported more frequently in people who had been vaccinated previously (p=0·04). No differences were seen for the other events, including redness (p=0·81), swelling (p=0·64), or fever (p=0·79; appendix p 3).

	Group 1	Group 2	Group 3	All groups
Redness				
Mild	8·8 (4·3–15·7)	5·3 (2·0–11·1)	8·4 (3·9–15·4)	7·5 (4·9–10·9)
Moderate	0 (0–3·2)	2·6 (0·5–7·5)	3·7 (1·0–9·3)	2·1 (0·8–4·3)
Severe	3·5 (1·0–8·8)	3·5 (1·0–8·7)	6·5 (2·7–13·0)	4·5 (2·5–7·3)
Swelling				
Mild	5·3 (2·0–11·2)	1·8 (0·2–6·2)	4·7 (1·5–10·6)	3·9 (2·1–6·6)
Moderate	0 (0–3·2)	4·4 (1·4–9·9)	5·6 (2·1–11·8)	3·3 (1·7–5·8)
Severe	4·4 (1·5–10·0)	6·1 (2·5–12·2)	6·5 (2·7–13·0)	5·7 (3·5–8·7)
Tenderness				
Mild	43·4 (34·1–53·0)	52·6 (43·1–62·1)	44·9 (35·2–54·8)	47·0 (41·6–52·5)
Moderate	42·5 (33·2–52·1)	35·1 (26·4–44·6)	42·1 (32·6–52·0)	39·8 (34·5–45·3)
Severe	4·4 (1·5–10·0)	5·3 (2·0–11·1)	2·8 (0·6–8·0)	4·2 (2·3–6·9)
Fever				
Mild	0 (0–3·2)	0·9 (0–4·8)	1·9 (0·2–6·6)	0·9 (0·2–2·6)
Moderate	0·9 (0–4·8)	0 (0–3·2)	0·9 (0–5·1)	0·6 (0·1–2·1)
Severe	0 (0–3·2)	0 (0–3·2)	0·0 (0–3·4)	0 (0–1·1)

Data are presented as percentage (95% CI). Redness and swelling were mild if 2·5–5 cm, moderate if 5·1–10 cm, and severe if more than 10 cm. Fever was mild if 38–38·4°C, moderate if 38·5–38·9°C, and severe if higher than 39°C.

Table 3: **Reported local reactions or fever according to group**

Discussion

We report, to our knowledge for the first time, the results of a randomised controlled trial investigating the timing of pertussis vaccination during pregnancy on cord blood pertussis antigen-specific IgG GMCs. We found that the equivalence criteria were met for anti-pertussis toxin and anti-pertactin antibody concentrations between babies born to mothers vaccinated at different time intervals, but there were higher anti-filamentous haemagglutinin antibody concentrations in babies born to mothers vaccinated at 28–32 weeks of gestation, compared with those vaccinated at less than 24 weeks of gestation.

These findings contrast with those of an observational study that concluded that vaccination during the second trimester was superior to vaccination during the third trimester.[12] Of note, this study included the whole of the third trimester (≥26 weeks) rather than the early third-trimester period used in our study (28–32 weeks). There have been several reports suggesting that vaccination in the early third trimester is superior to that of vaccination later in the third trimester,[8–11] which might explain this difference.

It is vital to understand the effect of timing of vaccination on the immunogenicity of these vaccines, but the aim of vaccinating women in pregnancy is not just to elicit the highest possible antibody concentrations, but to provide optimal protection to the whole population. Without a correlate of protection, it is difficult to be certain of the implications of the differences shown in anti-filamentous haemagglutinin antibody concentrations in our study, although other work has suggested that antibodies against filamentous haemagglutinin may not be bactericidal.[18] Furthermore, a UK observational study[19] has shown similar effectiveness against pertussis in infants following vaccination at different times in the second and third trimesters. These clinical data support our findings and suggest that the observed differences in anti-filamentous haemagglutinin antibody concentrations might not be of clinical significance.[19] Preterm infants are at an increased risk of severe pertussis,[20,21] but they are less likely to benefit from vaccination when this is administered later in pregnancy.[22] A UK observational study has shown that broadening the gestational age window at which antenatal pertussis vaccination is offered in the routine programme has been associated with a reduction in pertussis hospitalisations in preterm infants, although the number of preterm infants admitted both before and after the broadening of the window for vaccination was small.[23] We are unable to draw any conclusions about the effect of timing of maternal vaccination in infants born preterm because of the small numbers of such infants in our study. Another advantage of having a broad time window in which to offer vaccination is that it increases the opportunities for a vaccine to be administered.[23]

The reason for higher anti-filamentous haemagglutinin IgG concentrations at birth in neonates born to mothers vaccinated at 28–32 weeks of gestation appears to be a better initial response in women vaccinated in the third trimester (of statistical significance for group 3 [28–31 weeks+6 days] *vs* group 1 [≤23 weeks+6 days]) combined with a shorter interval to delivery resulting in less time for antibody waning. The combination of these factors means that antibody concentrations are subsequently higher through the third trimester and at delivery in both the mother and the baby. The differences in anti-filamentous haemagglutinin antibody concentrations at birth do not appear to reflect any differences in placental transfer ratios according to the timing of vaccination.

It is unclear why the maternal response to vaccination was greater in mothers vaccinated at 28–32 weeks of

gestation. An increased response to later vaccination has not previously been reported for pertussis; however, a meta-analysis of influenza vaccination showed that mothers vaccinated in a later trimester had a greater increase in haemagglutination inhibition titres (1·33 to 1·96 times greater) and higher haemagglutination inhibition titres in cord and neonate blood (1·21 to 1·64 times greater) compared with women vaccinated in an earlier trimester.[24]

Although there is no serocorrelate of protection for pertussis, higher antibody concentrations correlate with protection against disease. We followed Eberhardt and colleagues[12] in using an infant seropositivity calculation to assess the proportions of infants in each group who could be assumed to have protection until around 3 months, at which time they should be starting to benefit from their own vaccinations. We found no differences between the three study groups in this assessment of protection, suggesting that vaccination at any time between 19 weeks and 32 weeks might provide equivalent protection for young infants.

The increase in pertussis-specific IgG GMC at birth resulting from pertussis vaccination in pregnancy has been shown in some studies to result in blunting of antibody responses in infants following their own vaccinations.[25,26] The clinical significance of this finding is uncertain. In keeping with a meta-analysis,[25] we found that the timing of vaccination in pregnancy did not lead to a significant difference in antibody concentrations following primary vaccinations. However, there was notable attrition at the later study timepoints, so these findings should be further investigated with a larger sample size.

In many countries, there has been a programme for pertussis vaccination in pregnancy for several years. The existence of this programme means that an increasing number of women are receiving pertussis-containing vaccines in several pregnancies. There had previously been concerns that repeated administration of pertussis-containing vaccines might lead to more local and systemic side-effects, but subsequent work has reported that previous vaccination does not result in an increase in adverse events.[27,28] We identified an increase in reported local tenderness in women who had been vaccinated recently, but no increase in other local reactions or in the incidence of fever. We found that women who had recently been vaccinated, either in pregnancy or for other reasons, had higher antibody GMCs before vaccination. This finding is in keeping with previous work.[29] Because there is no correlate of protection, it is not possible to conclude whether participants who had previously been vaccinated might have had sufficient protection for themselves, or their babies, in the absence of another vaccination dose in pregnancy. However, data from the UK showed a lower effectiveness of 44% (95% CI 19–75) in those vaccinated only in a previous pregnancy, and therefore it can be concluded that vaccination in every pregnancy should still be advised.[19]

Although there was equivalence in higher anti-pertussis toxin and anti-pertactin antibody concentrations in the infant at birth, we did not investigate the avidity of these antibodies. It is possible that earlier vaccination results in higher avidity (or affects antibody function in another way), and this avidity is something which should be investigated further. As one of our exploratory objectives we are investigating the functional performance of the antibody using serum bactericidal antibody assays.

The follow-up phase of the study was substantially affected by the COVID-19 pandemic. The pandemic had a particular impact on the collection of infant samples, limiting the power of this aspect of the study (albeit a secondary objective). We used gestational age windows that were within the timeframe currently recommended in the UK, which means we are unable to comment on vaccination earlier in the second trimester or later in the third trimester. Information about which pertussis-containing vaccines participants had previously received was not collected, which means we are unable to comment on differences in response in women who had been primed with whole-cell versus acellular vaccine, or with a different acellular vaccine to the one they received in this trial. The vast majority of the participants will have received whole-cell pertussis vaccines in infancy.

We used broad eligibility criteria and recruited women attending hospital for routine antenatal care to recruit as representative a population as possible. However, the percentage of participants in this trial who were of non-White ethnicity was lower than in the pregnant UK population.

In the first randomised controlled trial to investigate the timing of pertussis vaccination in pregnancy on antibody concentrations in infants, we have shown that for two of the three antigens tested, there was equivalence of vaccination across the three time periods.

Considered together with recent vaccine effectiveness data, these results support the current guidance to vaccinate pregnant women any time between 16 weeks and 32 weeks of gestation.

OpTIMUM study group

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Contributors

AC, PTH, KLD, and CEJ conceived of the study. PTH was the lead applicant for funding and NA, AC, CEJ, AK, and KLD were coapplicants. AC, PTH, KLD, CEJ, GA, NA, SB, MC, BH, EJ, AK, MM, EP, MDS, and MV contributed to the study design. AC, SB, MC, HC, AE, VG, EJ, CEJ, KK, MM, EP, MDS, MV, and PTH were involved in the project administration. AC, KK, and VG did the data curation. MM, HC, and AE were responsible for sample testing. NA developed the statistical analysis plan and did the statistical analysis. AC, PTH, and NA accessed and verified the underlying data and drafted the manuscript. All authors reviewed and edited the manuscript and gave final approval for the version to be published.

Declaration of interests

GA and NA report that the Immunisation and Vaccine Preventable Diseases Division has provided vaccine manufacturers with postmarketing surveillance reports on pneumococcal and meningococcal infection, which the companies are required to submit to the UK Licensing Authority in compliance with their Risk Management Strategy. A cost recovery charge is made for these reports. CEJ has done studies on behalf of the University of Southampton and University Hospital Southampton NHS Foundation Trust funded by vaccine manufacturers, including Novavax, Moderna, Medicago, and Pfizer, but receives no personal funding for these activities. CEJ has served on advisory boards, data safety monitoring boards, or as a consultant for Moderna, MSD, Sanofi, Minervax, and Pfizer. MDS has acted as an investigator on behalf of the University of Oxford for studies funded or supported by vaccine manufacturers including GlaxoSmithKline, Pfizer, MCM vaccines, Novavax, AstraZeneca, and Janssen. MDS received no direct financial benefit for this work. From September, 2022 (after completion of this work), MDS became an employee of Medimmune and Moderna. PTH has conducted studies on behalf of St George's, University of London funded by vaccine manufacturers, including AstraZeneca, Novavax, Moderna, Valneva, Janssen, Minervax, and Pfizer, but receives no personal funding for these activities. All other authors declare no competing interests.

Data sharing

Data collected for the study, including individual (deidentified) participant data and a data dictionary defining each field in the set, can be made available for suitable applications submitted to the Chief Investigator (pheath@sgul.ac.uk). Additional related documents are also available on request.

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