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# COVID-19 vaccination before or during pregnancy results in high, sustained maternal neutralizing activity to SARS-CoV-2 wild-type and Delta/Omicron variants of concern, particularly following a booster dose or infection

Aniza P. Mahyuddin<sup>1,#</sup>, Hannah L.F. Swa<sup>2,#</sup>, Ruifen Weng<sup>2,#</sup>, Jingxian Zhang<sup>2</sup>, Janice P. Dhanaraj<sup>2</sup>, Binny P. Sesurajan<sup>1</sup>, Mary Rauff<sup>1,3</sup>, Pradip Dashraath<sup>1,3</sup>, Abhiram Kanneganti<sup>3</sup>, Rachel Lee<sup>3</sup>, Lin-Fa Wang<sup>4,5</sup>, Barnaby E. Young<sup>5,6,7,8,9</sup>, Paul A. Tambyah<sup>9,10</sup>, David C. Lye<sup>5,6,7,8,9</sup>, Louis Y.A. Chai<sup>9,10</sup>, Sidney Yee<sup>11</sup>, Mahesh Choolani<sup>1,3</sup>, Citra N.Z. Mattar<sup>1,3,\*</sup>

<sup>1</sup> Department of Obstetrics and Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

<sup>2</sup> Diagnostics Development Hub, Agency for Science, Technology and Research, Singapore, Singapore

<sup>3</sup> Department of Obstetrics and Gynaecology, National University Hospital, National University Health System, Singapore, Singapore

<sup>4</sup> Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, Singapore

<sup>5</sup> The Programme for Research in Epidemic Preparedness and Response (PREPARE), National Centre for Infectious Diseases, Singapore, Singapore

<sup>6</sup> National Centre for Infectious Diseases, Singapore, Singapore

<sup>7</sup> Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore, Singapore

<sup>8</sup> Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore

<sup>9</sup> Infectious Diseases Translational Research Program, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

<sup>10</sup> Division of Infectious Diseases, Department of Medicine, National University Hospital, National University Health System, Singapore, Singapore

<sup>11</sup> Innovation and Enterprise, Agency for Science, Technology and Research, Connexis North Tower, Singapore, Singapore

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## ABSTRACT

**Objectives:** To investigate multi-dose and timings of COVID-19 vaccines in preventing antenatal infection. **Design:** Prospective observational study investigating primary vaccinations, boosters, antenatal COVID-19 infections, neutralizing antibody (Nab) durability, and cross-reactivity to Delta and Omicron variants of concern (VOCs).

**Results:** Ninety-eight patients completed primary vaccination pre-pregnancy (29.6%) and antenatally (63.3%), 24.2% of whom had antenatal COVID-19, while 7.1% were unvaccinated (28.6% had antenatal COVID-19). None had severe COVID-19. Pre-pregnancy vaccination resulted in vaccination-to-infection delay of 23.3 weeks, which extended to 45.2 weeks with a booster, compared to 16.9 weeks following antenatal vaccination ( $P < 0.001$ ). Infections occurred at 26.2 weeks gestation in women vaccinated pre-pregnancy compared to 36.2 weeks gestation in those vaccinated during pregnancy ( $P < 0.007$ ). The risk of COVID-19 infection was higher without antenatal vaccination (hazard ratio [HR] 14.6,  $P = 0.05$ ) and after pre-pregnancy vaccination without a booster (HR 10.4,  $P = 0.002$ ). Antenatal vaccinations initially led to high Nab levels, with mild waning but subsequent rebound. Significant Nab enhancement occurred with a third-trimester booster. Maternal-neonatal Nab transfer was efficient (transfer ratio  $> 1$ ), and cross-reactivity to VOCs was observed.

**Conclusion:** Completing vaccination during any trimester delays COVID-19 infection and maintains effective neutralizing activity throughout pregnancy, with robust cross-reactivity to VOCs and efficient maternal-neonatal transfer.

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\* Corresponding author: Citra NZ Mattar, Department of Obstetrics and Gynaecology, NUS Yong Loo Lin School of Medicine, National University of Singapore, 1E Kent Ridge Road, NUHS Tower Block, Level 12, Singapore 119228, Singapore.

E-mail address: [citramattar@nus.edu.sg](mailto:citramattar@nus.edu.sg) (C.N.Z. Mattar).

# Joint lead authors.

## Introduction

As we enter a phase of endemicity, COVID-19 remains a substantial public health burden [1,2]. Since 2022, multiple lineages within the SARS-CoV-2 Omicron variant family have continued to drive COVID-19 infections globally, with new Omicron XBB subtypes likely to emerge through ongoing viral evolution [3]. In Singapore, steadily rising rates of COVID-19 infections, hospitalizations, and intensive care unit admissions have been attributed to BA.2.86-sublineage JN.1, which currently accounts for >60% of local infections, coupled with waning population immunity and increased exposure through global travel [2]. In the United States, Omicron EG.5 and HV.1 are currently responsible for half of all reported cases [4]. Notably, Omicron variants are known to evade previously acquired vaccine- and infection-induced immunity against COVID-19 [5], though these sublineages are unlikely to cause more severe disease than other circulating variants [6]. Protection from clinical infection afforded by vaccination and hybrid immunity is likely to eventually wane [7], leading to the need for annual booster vaccinations [8].

COVID-19 infection in pregnancy is associated with an increased risk of maternal morbidity (intensive care unit admissions, need for mechanical ventilation, and maternal mortality) and antenatal complications (preterm birth, miscarriage, stillbirth, fetal growth restriction, and placental abruption), particularly among symptomatic and unvaccinated individuals [9,10]. Guidelines strongly recommend SARS-CoV-2 messenger RNA (mRNA) or protein subunit primary vaccination series and booster doses during pregnancy to reduce these complications [11,12]. Although pregnant individuals were initially excluded from COVID-19 vaccine trials [13], nonrandomized observational studies have since shown no increased risk for adverse effects, including miscarriage and congenital abnormalities [14]. Maternal vaccination also establishes passive immunity in infants up to 6 months of age through placental transfer of antibodies into cord blood [15]. However, an understanding of how maternal immune adaptations across pregnancy influence the efficacy of COVID-19 vaccines is lacking [16]. This information is critical in advising the timing of maternal SARS-CoV-2 vaccination for public health policy. Further, in considering the benefit and optimal timing of booster doses, information on the waning of vaccine effectiveness in mothers inoculated before pregnancy is lacking. According to official statistics, an estimated 84–90% of the Singapore population in the 18–49 year age range most relevant to maternity has vaccine-induced protection (primary vaccination series and booster) as of July 2023 [2]. In this study, we investigated the effectiveness of multiple doses of COVID-19 vaccines in preventing infection among pregnant individuals vaccinated before, during, and after pregnancy. In addition, we prospectively characterized maternal neutralizing antibody durability in each trimester of pregnancy and in neonatal cord blood at delivery, including the impact of hybrid immunity (through vaccination and natural SARS-CoV-2 infection) to determine the optimum timing of vaccination during the antenatal period.

## Materials and methods

### Study design, setting, and participants

This prospective observational study was conducted at the National University Hospital Singapore, under Domain-Specific Review Board approval 2012/00917. Pregnant women were recruited from the outpatient antenatal clinics and inpatient wards, before or after COVID-19 vaccination with two doses of either BioNTech/Pfizer BNT162b2/Comirnaty, Moderna mRNA-1273/SpikeVax or Sinopharm BBIBP-CorV from August 24, 2021, to May 24, 2022. Primary vaccination was defined as the completion of two vaccine

doses 1 month apart, while booster was defined as a third dose following primary vaccination. The Ministry of Health, Singapore (MOH), recommends three mRNA vaccine doses as minimum protection [17]. Study participants were classified according to their vaccination status as primary vaccination (1) completed prepregnancy, (2) completed antenatally during first (T1), second (T2), or third (T3) trimesters, and (3) unvaccinated (vaccination initiated after childbirth).

The recruitment and surveillance period covered the Delta Variant (June–November 2021) and Omicron Variant (November 2021–2023) Periods [18]. Women  $\geq 21$  years were recruited at all stages of pregnancy and of any vaccination status at recruitment. All pregnant women with singleton or multiple fetuses were eligible. Those who refused participation, refused vaccination, had COVID-19 before pregnancy (testing positive on COVID-19 home test kits), intended to deliver at another hospital, and declined additional blood sample collection were excluded. Participants gave informed written consent. Data and blood samples were collected from mothers at multiple time points coinciding with routine antenatal investigations at gestational weeks 10–12 (designated T1), 18–22 (T2), 24–28 (late T2), 34–36 (T3), at delivery (D0), and at two and six postpartum months. Women were recruited at any gestational age (GA) during the antenatal review, and blood was collected at the nearest time point specified by the protocol to standardize sampling intervals. Umbilical cord blood (UCB) was collected at D0.

### Detection of SARS-CoV-2 neutralizing antibody

Blood samples were collected in sodium EDTA anticoagulated tubes, centrifuged at 1000xG for 10 minutes, and plasma assayed using cPass SARS-CoV-2 Neutralization Antibody Detection Kit or SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT, GenScript Biotech, Singapore) according to the manufacturer's protocol. Briefly, diluted samples were incubated with RBD-HRP (receptor binding domain-horseradish peroxidase) at 37°C for 30 minutes to form neutralizing antibody-RBD-HRP complexes, incubated in hACE2 (human angiotensin-converting enzyme 2)-coated plates at 37°C for 15 minutes, and washed with buffer to remove neutralizing antibody-RBD-HRP complexes, leaving unbound RBD-HRP and nonneutralizing antibody-RBD-HRP complexes captured on the plate. Bound complexes were incubated with TMB (3,3',5,5'-Tetramethylbenzidine) substrate solution for 15 minutes, stop solution added, and colorimetry measured via absorbance reading at 450 nm. Detection of isotype-independent neutralizing antibodies (Nab) against wild-type virus, and Delta and Omicron variants was determined using SARS-CoV-2 wild-type, Delta or Omicron variant Spike protein RBD-HRP (GenScript Biotech), respectively, with the same test kit. Qualitative titers of anti-SARS-CoV-2 Nab were derived by colorimetry absorbance at 450 nm, from which the percentage (%) inhibition was derived according to the manufacturer's instructions. The sample was read as negative if Nab was at <30% inhibition. Nab waning ratio was calculated as last Nab (at time point-of-interest)/initial Nab).

### Statistical analysis

Patient biodata and timings of vaccine administration and COVID-19 infection (reported as mean  $\pm$  standard deviation, SD), were compared between vaccination status subgroups using Student's *t*-test (GraphPad Prism 10.0 for Windows 64-bit Version 10.0.2 (232) (GraphPad Software; Boston; USA, [www.graphpad.com](http://www.graphpad.com); 2023). Multivariate logistic and linear regression analyses were performed to study the association between COVID-19 infection and the timing of vaccination, gestation at delivery, age, ethnicity, comorbidities, mode of delivery, birth weight, and gender. Kaplan–Meier and Cox Proportional Hazards Models survival

analyses were performed using R Version 4.3.2 (R Core Team; Vienna; Austria; 2023), utilizing the survival package along with “survminer” and “ggplot2” for visualization (<https://rpkgs.datanovia.com/survminer/>) to determine the likelihood of COVID-19 infection during pregnancy and the intervals between primary vaccination and infection (expressed as median, range). The data was censored at the date of delivery (numbers at risk at each time-point are shown in Supplementary Figure 1). Nab levels (median, range), waning patterns, and correlations are represented by box-plots and scatter plots generated by “ggpubr” R package (<https://cran.r-project.org/web/packages/ggpubr/index.html>), version 0.4.0. Student’s *t*-test was used to compare differences between groups for continuous variables: percentage inhibition (Nab titers) and waning ratios. Pearson correlation coefficient was used to assess cross-reactivity of SARS-CoV-2 Nab to Delta and Omicron RBDs (R stats package (<https://www.r-project.org/>), version 4.0.3). Significance was set at *P*-value <0.05. 95% confidence intervals (CI) and hazard ratios (HR) were stated where applicable.

## Results

### Participant characteristics

A total of 104 pregnant women were recruited from August 24, 2021, to May 24, 2022. Six women were eventually lost to follow-up before study completion. Out of the 98 women enrolled in the study, 29.6% (*n* = 29) were in the preconception phase, 13.3% (*n* = 13) were in the first trimester, 38.8% (*n* = 38) were in the second trimester, 11.2% (*n* = 11) were in the third trimester, and 7.1% (*n* = 7) were in the postpartum period. We collected multiple blood samples from 98 participants at predetermined time points over an 8–52-week period with the final sample collected on March 30, 2023. Of these 98 women, 86 received BNT162b2/Comirnaty, 11 received mRNA-1273/SpikeVax, and one received BBIBP-CorV. While all women provided a minimum of two blood samples, the majority provided between four and seven blood samples over a period ranging between 24 and 52 weeks from recruitment.

Vaccination timings and pregnancy outcomes are summarized in Supplementary Table 1. Twenty-nine of 98 women (29.6%; *n* = 29) completed primary vaccination at a mean of  $9.2 \pm 13.4$  weeks (w) prepregnancy and received booster doses during pregnancy at GA  $18.7 \pm 8.5$  w. Sixty-two of 98 women (63.3%; *n* = 62) received primary vaccination during pregnancy; 21.0% (*n* = 13), 61.3% (*n* = 38), and 17.7% (*n* = 11) were vaccinated at T1, T2, and T3, respectively, with booster doses received at GA  $33.2 \pm 10.9$  w, or at  $8.8 \pm 5.1$  w and  $22.2 \pm 5.7$  w postpartum, respectively. Finally, 7.1% women (*n* = 7) completed primary vaccination at  $5.6 \pm 2.4$  postpartum weeks and received booster doses at  $12.3 \pm 0.0$  postpartum weeks. Pregnancy outcomes were similar across all three groups, with delivery around GA ~39 w and an overall cesarean delivery rate of 19.4%. There was no significant difference in birth weights.

### Vaccination timing, duration of protection, and COVID-19 infection

Overall, 24 of 98 women (24.5%; *n* = 24) had COVID-19 infections during pregnancy; of these women, 50.0% (*n* = 12) completed prepregnancy vaccination, and 41.7% (*n* = 10) were vaccinated during pregnancy (one in T1, eight in T2, and none in T3) at an average of  $13.1 \pm 7.5$  w prepregnancy and at GA  $21.3 \pm 3.9$  w, respectively, while the remaining two patients (8.3%; *n* = 2) were unvaccinated during pregnancy. Comparing women with antenatal COVID-19 and no infection, there were no significant differences in vaccination timing among those inoculated before, during or after pregnancy, and among mothers receiving boosters postpartum.

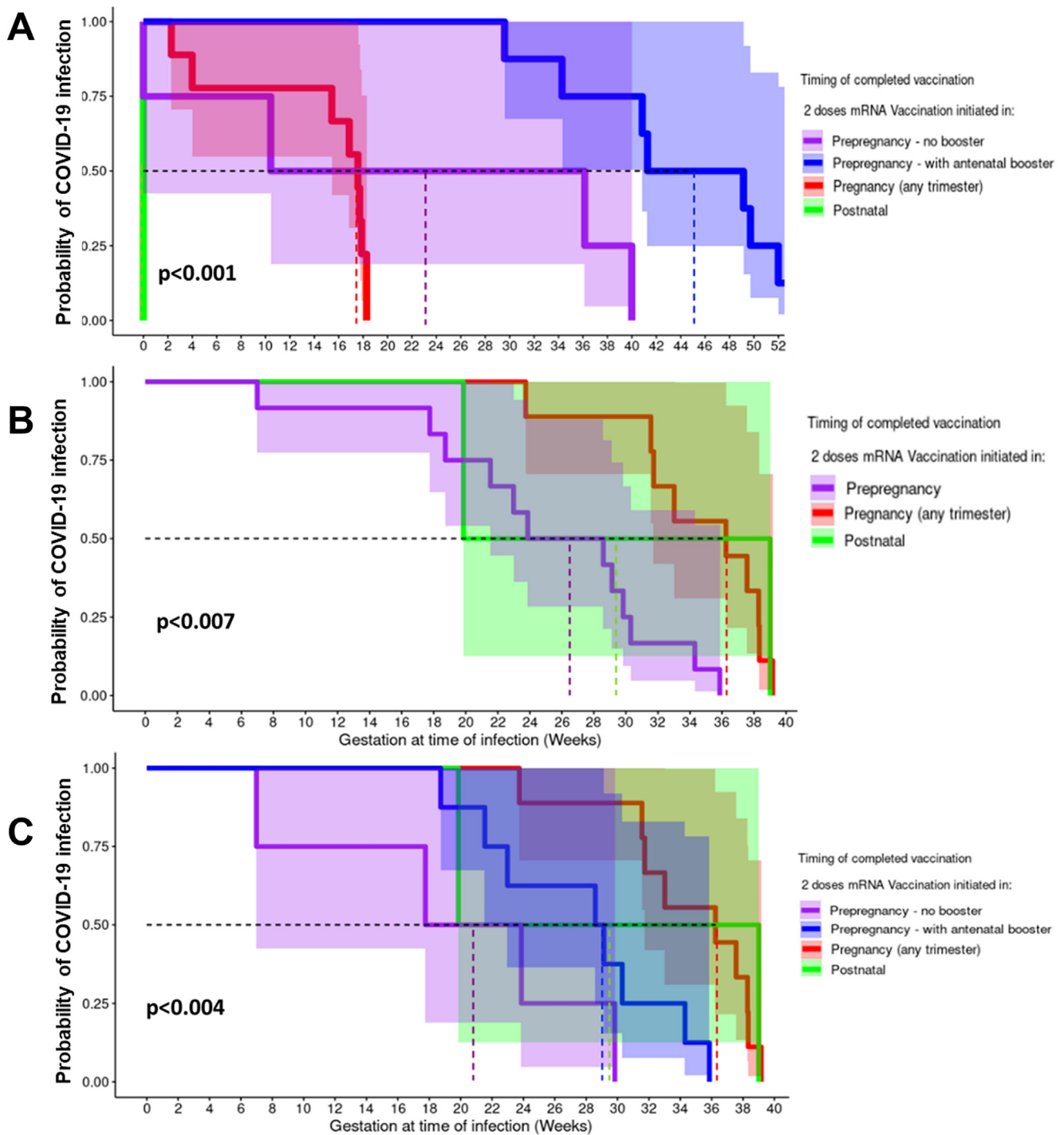
However, COVID-19-infected mothers were given boosters significantly earlier at GA  $16.4 \pm 7.9$  w compared to a later booster dose at GA  $25.1 \pm 9.5$  w received by uninfected mothers (*P* = 0.02, Table 1). Of these 24 patients, 41.2% (*n* = 10) had mild to moderate respiratory symptoms and fever, and 58.3% (*n* = 14) had minimal symptoms with none requiring intensive care or developing severe disease. Multivariate regression analysis showed that COVID-19 infection occurred at GA  $29.1 \pm 8.41$  w, and the mean intervals between primary vaccination and COVID-19 infection, and COVID-19 infection and delivery, were  $26.9 \pm 17.1$  w and  $12.4 \pm 12.2$  w, respectively. There was no association between COVID-19 infection and maternal age, body mass index, pregnancy duration, delivery mode, birthweight, and neonatal Apgar scores (data not shown).

Following prepregnancy primary vaccination, the median interval between completion and antenatal COVID-19 infection was 23.3 w (0–40.0 w, *n* = 4) without a booster dose, and this interval was extended to a median interval of 45.2 w (29.6–53.7 w, *n* = 8) if a booster was given in any trimester. During pregnancy inoculation produced the shortest interval between vaccination and antenatal COVID-19 infection at 16.9 w (2.3–18.3 w, *n* = 9) compared to other vaccination time points (*P* < 0.001, Figure 1a). Prepregnancy vaccination (regardless of booster) resulted in COVID-19 at a significantly earlier GA of 26.2 w (7.0–35.9 w, *n* = 12, *P* < 0.007) compared to a GA of 36.2 w (23.7–39.2 w, *n* = 9), *P* < 0.007) with antenatal vaccination in any trimester (Figure 1b). Women completing primary vaccination during pregnancy acquired COVID-19 infection significantly later at GA of 36.2 w (23.7–39.2 w, *n* = 9), respectively, compared to women with prepregnancy vaccination receiving a booster at any trimester (28.8 w (18.7–35.9 w), *n* = 8, *P* < 0.004, Figure 1c). Unvaccinated pregnant women who received postnatal vaccinations had COVID-19 infection at a median GA of 29.5 w (19.9–39.0 w, *n* = 2). Cox proportional hazards regression modeling demonstrated significantly elevated infection risk without vaccination during pregnancy (HR = 14.6, 95% CI = 0.8–244.4; *P* = 0.05) and following prepregnancy vaccination without booster (HR = 10.4, 95% CI = 2.3–46.1; *P* = 0.002).

### Duration and waning of SARS-CoV-2 neutralizing antibody titers

Women receiving two doses of BNT162b2/Comirnaty or mRNA-1273/SpikeVax achieved ~95.0% inhibitory activity against SARS-CoV-2 wild-type RBD at 1–3 months from vaccine completion. Some degree of waning was observed in the first month following completion of primary vaccination in all patients who did not acquire antenatal COVID-19. Lower initial inhibitory activity resulted in a greater magnitude of waning over the study period. Nab were generally maintained within a median waning window of 1.5–22.0% of the initial levels over the intervals between sample collection and primary vaccination ranging from 5 to 30 w (Figure 2a). The greatest waning reached a median of 22.0% around 21–24 w, with partial recovery of Nab approaching 25–30 w postvaccination (differences not significant). In contrast, minimal waning was observed across the same time points in women with an additional booster (Supplementary Figure 2). Maintenance of neutralizing activity was studied according to vaccination timing in uninfected pregnant women with completed primary vaccination.

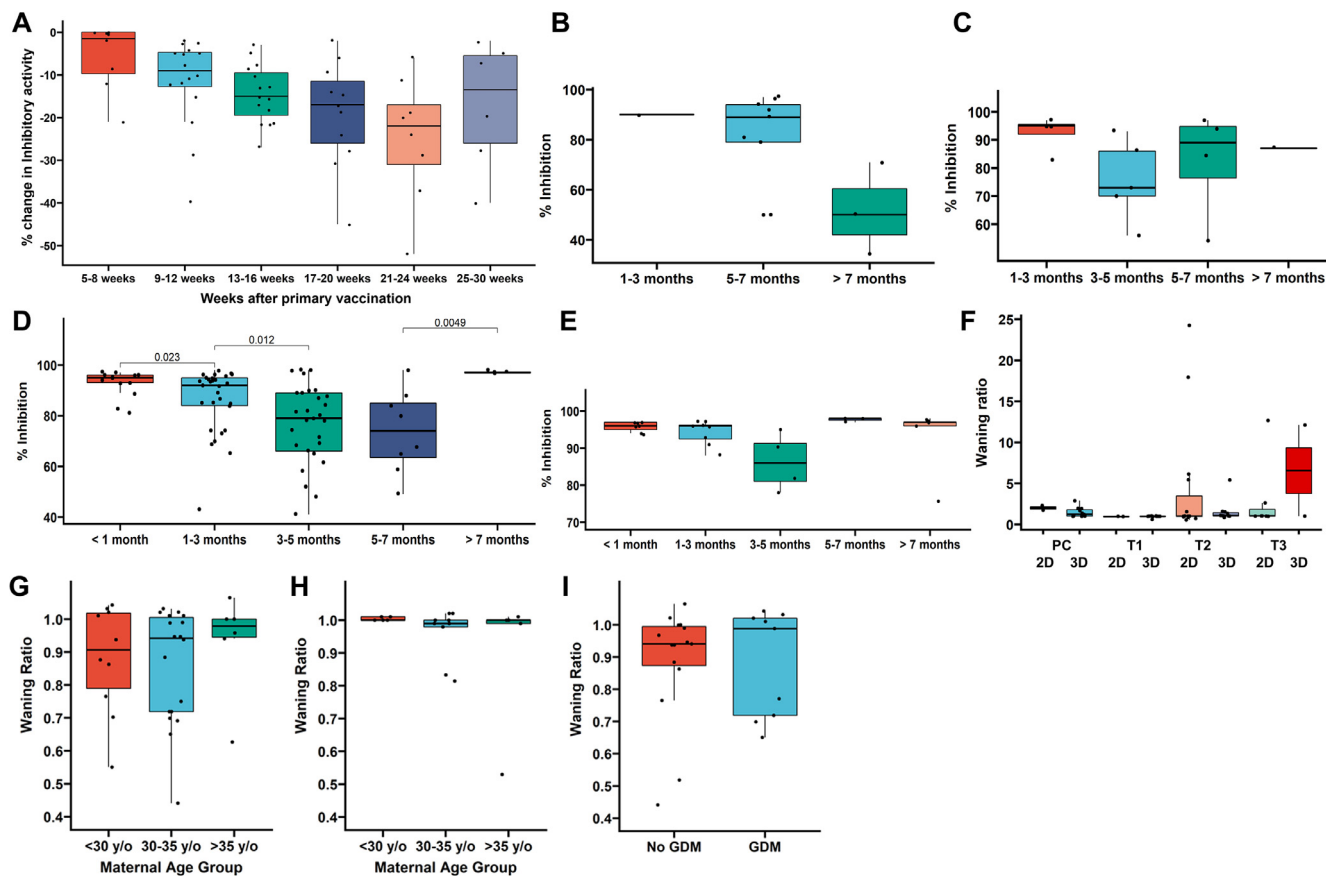
Vaccination in all trimesters resulted in high levels of surrogate viral Nab which waned mildly across the cohort for all three groups but rebounded for those completing primary vaccination across all trimesters. Prepregnancy vaccination was associated with Nab titers ~90.0% (range 89.0–90.0%) for the first 5–7 months, falling to 50.0% at 7 months onwards (Figure 2b). T1 vaccination was associated with initial Nab of ~95.0% (83.0–97.0%), waning to 73.0% (56.0–93.0%) at 3–5 months, followed by recovery to 89.0% (54.0–97.0%) from 5 to 7 months onwards (Figure 2c). Vaccination in T2 produced initial Nab titers of ~95.0% (81.0–97.0%),



**Figure 1.** Kaplan-Meier survival curves demonstrating the probability of antenatal COVID-19 infection related to the completion of primary vaccinations and booster doses. (A) Completion of primary vaccination before pregnancy without booster was associated with infection at a median of 23.3 weeks following completion, and with booster (at any time of pregnancy) 45.2 weeks ( $n = 23$ ,  $P < 0.001$ ). Not being vaccinated during pregnancy (only receiving postnatal vaccination) was associated with increased risk of COVID-19 infection. Primary vaccination during pregnancy (any trimester) was associated with infection occurring at a median of 16.9 weeks ( $n = 9$ ,  $P < 0.001$ ) after vaccination. (B) Completion of vaccination before pregnancy was associated with increased risk of COVID-19 infection during pregnancy at median gestation of 26.2 weeks ( $n = 12$ ,  $P < 0.007$ ), while completion of vaccination during pregnancy (any trimester) was associated with increased risk of COVID-19 infection during pregnancy at median gestation of 36.2 weeks ( $n = 9$ ,  $P < 0.007$ ). (C) Primary vaccination before pregnancy without booster was associated with a higher risk of COVID-19 infection during pregnancy at a median gestation of 21.5 weeks ( $n = 4$ ,  $P < 0.004$ ), compared to prepregnancy primary vaccination with antenatal booster ( $n = 8$ ,  $P < 0.004$ ), and primary vaccination during pregnancy ( $n = 9$ ,  $P < 0.004$ ).

**Table 1**  
Comparison of women who had COVID-19 infections during pregnancy and vaccination doses.

Number of women, <i>n</i>	COVID-19 infection in pregnancy		No COVID-19 infection in pregnancy		<i>p</i> -value	95% CI of means
	<i>n</i> (%)	Weeks	<i>n</i> (%)	Weeks		
<b>Completion of primary vaccination (2 doses)</b>						
Prepregnancy (weeks before conception)	12 (50.0%)	13.1 ± 7.5	13 (17.6%)	10.9 ± 9.6	0.54	-8.48 to 3.34
During pregnancy (gestational age)	10 (41.7%)	21.3 ± 3.9	56 (75.7%)	23.9 ± 9.1	0.39	-5.05 to 9.32
Postpartum (weeks after delivery)	2 (8.3%)	6.0 ± 4.2	5 (6.8%)	5.7 ± 2.2	0.91	-5.65 to 6.22
<b>Administration of Booster Dose (after primary vaccination pre- or during pregnancy)</b>						
During pregnancy	9 (37.5%)	16.4 ± 7.9	22 (40.5%)	25.1 ± 9.5	0.02	-15.99 to -1.31
Postpartum	2 (8.3%)	13.4 ± 0.5	19 (25.7%)	10.7 ± 6.8	0.60	-7.64 to 12.94
Gestational age at delivery, weeks		39.0 ± 1.8		39.3±1.0	0.33	-0.88 to 0.30



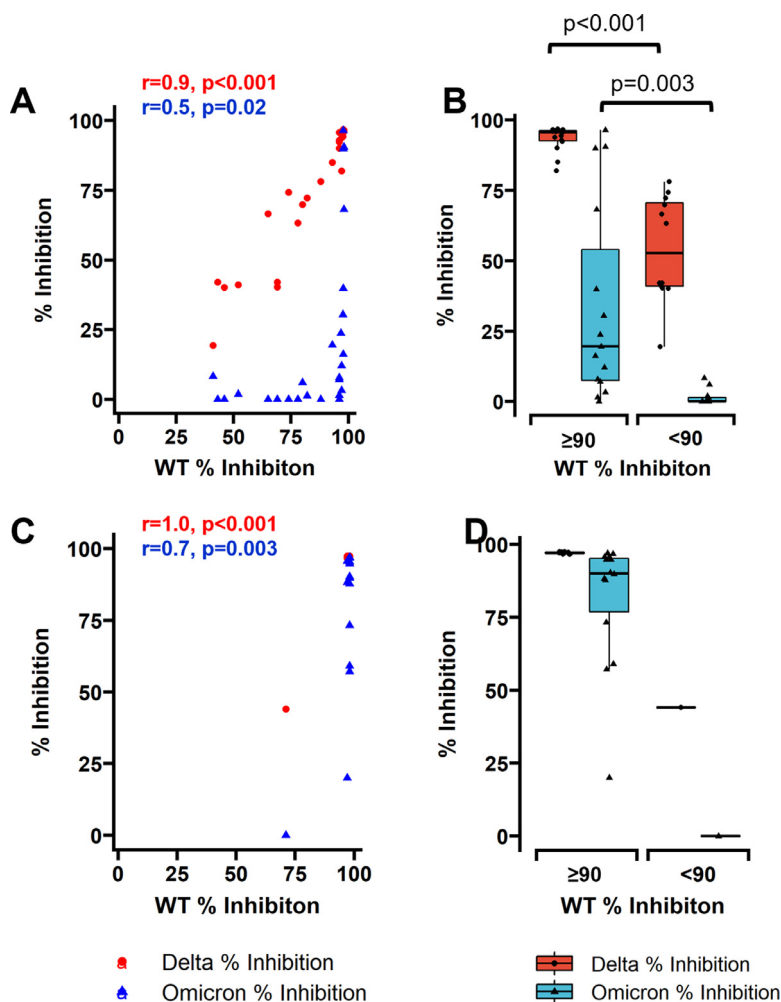
**Figure 2.** Neutralizing Antibody durability to SARS-CoV-2 vaccination and infection. (A) Nab titers were generally maintained within median waning of 1.5–22.0% of the initial levels over periods of time between blood samples collection and primary vaccination ranging from 5 to 30 weeks. Nab levels waned by 1.5% (median) at 5–8 weeks postvaccination, to 22.0% (median) around 21–24 weeks (nadir) with partial recovery of Nab titers when approaching 25–30 weeks postvaccination. These differences were not significant. (B) Vaccination was initiated prepregnancy. (C) Vaccination was initiated in T1. (D) Vaccination was initiated in T2. (E) Vaccination initiated in T3. (F) Variation of Nab titers among the different vaccination schedules. (G) Mothers who had completed primary vaccination showed similar waning Nab patterns across the 3 age groups. Individual mothers' Nab levels showed waning of up to 56.0% (lowest waning ratio 0.44) in 30–35-year-old women (no significant differences). (H) With an additional booster, a lower waning pattern was observed, and most individuals maintained their original Nab titers (waning ratio 1.0). One mother of >35 years old showed a drop in inhibition to 53.0% of initial activity (no statistical significance). (I) Nab waning profile did not differ between women with or without gestational diabetes. 2D, primary vaccination; 3D, primary vaccination & booster; GDM, gestational diabetes mellitus; T1, first trimester; T2, second trimester; T3, third trimester.

which dropped to 92.0% at 1–3 months (43.0–98.0%,  $P = 0.023$ ), and further to 74.0% (41.0–98.0%) at 3–7 months postvaccination. In women monitored for >7 months, Nab titers recovered to 97.0% (97.0–98.0%,  $P = 0.005$ , Figure 2d). Vaccination initiated in T3 produced Nab titers of ~96.0% for the first 3 months, waning to 86.0% (78.0–98.0%) between 3 and 5 months and returning to 97.0% from 5 to 7 months onwards (Figure 2e). Completing primary vaccination prepregnancy or during T1, with or without a booster, resulted in minimal waning (waning ratio = last Nab/initial Nab, range 0.97–2.03) with no differences between groups. Enhancement of Nab titers (waning ratio 6.57) was observed in women complet-

ing primary vaccination with a booster in T3 (Figure 2f). Individual mothers' Nab levels showed a wide range of waning ratios of up to 56.0% (Figure 2g-i).

*Cross-reactivity of SARS-CoV-2 neutralizing activity to the receptor binding domains of Delta and Omicron variants of concern*

In uninfected and fully vaccinated pregnant women, a strong correlation was observed between surrogate Nab titers to wild-type and Delta RBD ( $r = 0.95$ ). In contrast, poor correlation was observed between wild-type and Omicron RBD neutralizing



**Figure 3.** Cross-reactivity of SARS-CoV-2 neutralizing antibody to the receptor binding domains (RBD) of Delta and Omicron Variants of Concern. (A) A strong correlation was observed between surrogate Nab titers to wild-type and Delta RBD in uninfected women who had either 2 or 3 vaccine doses. (B) Inhibitory activity to wild-type RBD compared to inhibitory activity to Delta RBD and Omicron RBD. (C) COVID-19 infection in mothers who had completed vaccination resulted in a higher inhibitory activity to wild-type and Delta RBDs and stronger inhibition to Omicron RBD. (D) Inhibitory activity of  $>90.0\%$  to wild-type RBD results in  $>90\%$  inhibition to Delta RBD and  $>90.0\%$  median inhibition to Omicron RBD, in contrast to lower  $<90.0\%$  inhibition to wild-type RBD which results in  $44.0\%$  inhibition to Delta and  $0\%$  inhibition to Omicron RBDs.

activities ( $r = 0.46$ , Figure 3a). In general, Nab  $\geq 90\%$  ( $n = 15$ ) to wild-type RBD produced  $96.0\%$  inhibition ( $82.0\text{--}96.8\%$ ) to Delta RBD, but only  $20.0\%$  inhibition ( $0.0\text{--}96.5\%$ ) to Omicron RBD, though in three mothers' inhibitory activity to Omicron was  $\geq 70.0\%$  (Figure 3a,b). There were significantly higher Nab to both Delta ( $P < 0.001$ ) and Omicron RBDs ( $P = 0.003$ ) in women with Nab  $\geq 90\%$  to wild-type RBD compared to women with  $<90\%$  ( $n = 12$ ) inhibitory activity. COVID-19 infection promoted stronger cross-reactivity to Delta (neutralizing activity  $97.0\%$ , range  $96.6\text{--}97.5\%$ ,  $r = 1.00$ ) and Omicron RBD ( $90.0\%$ , range  $20.0\text{--}96.9\%$ ,  $r = 0.71$ ) if Nab to wild-type RBD was  $\geq 90.0\%$  ( $n = 14$ ) (Figures 3c,d), while  $<90\%$  ( $n = 1$ ) Nab produced low inhibition to Delta and Omicron RBDs.

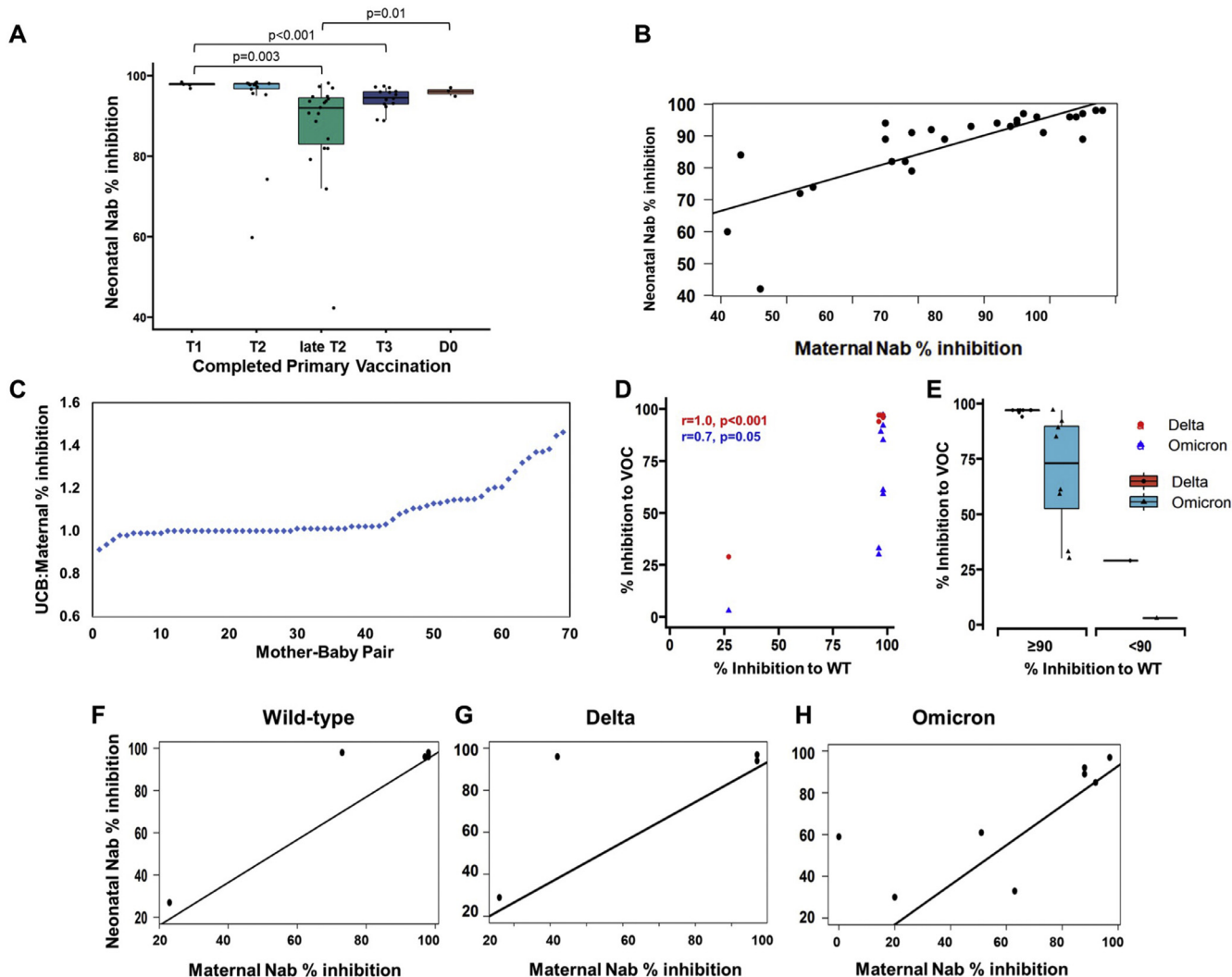
#### Neonatal transfer of immunity

We examined 70 mother-baby dyads where paired maternal and UCB samples taken at delivery were assayed. While primary vaccination at any trimester resulted in surrogate viral Nab titers of  $\sim 92\text{--}95\%$  in UCB, vaccination in T1 produced marginally higher UCB Nab than T2 or T3 vaccination (Figure 4a). Vaccination in T2 showed the strongest correlation between maternal and neonatal Nab titers at delivery (Pearson  $r = 0.79$ ) over a broad maternal Nab range of  $43.0\text{--}98.0\%$  (Figure 4b). Successful neonatal transfer of Nab

was assessed by calculating the transfer ratio (TR, % inhibition in UCB: maternal blood), with a TR  $>1.0$  indicating efficient transfer. Overall, the TR was just  $>1.0$ , with median UCB inhibition at  $96.0\%$  ( $42.0\text{--}98.0\%$ ) vs maternal inhibition of  $95.0\%$  ( $41.0\text{--}98.0\%$ ,  $n = 70$ ).  $14.3\%$  ( $n = 10$ ) had TR  $<1.0$ , with UCB and maternal inhibition at  $95.5\%$  ( $42.0\text{--}97.0\%$ ) and  $97.0\%$  ( $46.0\text{--}98.0\%$ ), respectively. Of the mother-baby dyads with TR  $> 1.0$ ,  $51.4\%$  ( $n = 36$ ) had similar UCB and maternal inhibition of  $\sim 98.0\%$ . Despite a waning of maternal immunity at delivery in 24/70 mothers ( $67.5\%$ , range  $41.0\text{--}86.0\%$ ), UCB inhibition in these pairs persisted at  $87.3\%$  ( $60.0\text{--}98.0\%$ ), maintaining a TR of  $1.1$  to  $\geq 1.4$  (Figure 4c). Birthweight and neonatal gender were not significant factors in maternal-fetal inhibitory antibody transfer (data not shown). Anti-wild-type neonatal Nab from nine pregnancies affected by COVID-19 correlated strongly with neonatal Nab inhibiting Delta and Omicron (Figure 4d,e) and with maternal Nab to all three variant RBDs (Figure 4f-h).

#### Discussion

We show that primary vaccination before or during pregnancy resulted in  $>90\%$  neutralizing activity to wild-type SARS-CoV-2 RBD within the first 3 months following completion, with Nab levels waning mildly at 3-5 months but generally remaining  $\sim 90\%$  and persisting for  $>7$  months, though this did not apply to



**Figure 4.** Maternal-to-fetal transfer of neutralizing antibodies at delivery. (A) Completion of primary vaccination by 24–28 weeks (late T2,  $n = 16$ ) or by 36 weeks (T3,  $n = 19$ ) of gestation resulted in lower neonatal Nab at delivery compared to completion by end of T1 ( $92.0 \pm 13.0\%$ ,  $P = 0.003$  and  $94.5 \pm 4.0\%$ ,  $P < 0.001$ , respectively), and lower Nab compared to vaccination just before D0 ( $P = 0.01$ ). (B) Vaccination initiated in T2 showed strong correlation between maternal and neonatal Nab titers at delivery. (C) The maternal-neonatal transfer ratio (TR) was greater than 1.0 for 85.7% ( $n = 60$ ) of cases, while 14.3% ( $n = 10$ ) had a TR less than 1.0. (D) High correlation was observed between SARS-CoV-2 Nab in neonates whose mothers had COVID-19 infections, and inhibitory activity to Delta and Omicron RBDs in umbilical cord blood (UCB) ( $r=1$  and  $r=0.68$  respectively). (E) High inhibition to neonatal wild-type RBD  $\geq 90.0\%$  ( $n = 8$ ) resulted in median 95.0% (94.0–97.0%) and median 74.0% (30.0–97.0%) inhibition to Delta and Omicron RBDs, respectively, in UCB, whereas  $<90.0\%$  ( $n = 1$ ) inhibition to wild-type RBD produced 29.0 and 3.0% inhibition to Delta and Omicron RBDs, respectively. (F,G) High correlation was observed between UCB and maternal Nab inhibition of wild-type RBD (Pearson  $r = 0.94$ ,  $P < 0.001$ ,  $n = 9$ ), with moderately-high correlation of inhibitory activity to Delta variant RBD of UCB and maternal Nab (Pearson  $r = 0.77$ ,  $P = 0.016$ ,  $n = 9$ ). (H) There was moderately strong correlation between UCB and maternal Nab inhibitory activity to Omicron variant RBD (Pearson  $r = 0.80$ ,  $P < 0.002$ ,  $n = 9$ , h).

inhibition against Omicron variants of concern (VOC). Primary vaccination completed in T1 or T2 resulted in the shortest interval between vaccination and infection, while booster doses in any trimester lengthened this interval, resulting in most COVID-19 infections being deferred to T3 (as opposed to occurrence in earlier trimesters). This finding suggests that early T3 may be the ideal time for booster vaccination compared to the relatively altered immune system periods of T1 and T2. Hybrid immunity following COVID-19 infection in vaccinated women resulted in high levels of surrogate Nab to wild-type virus and robust cross-inhibition to Delta and Omicron variants. Neonatal Nab transfer was efficient following vaccinations in all trimesters and was protective against Delta and Omicron strains.

Most countries currently have policies permitting or recommending primary immunizations with mRNA or vector-derived vaccines at any stage of pregnancy, including of women previously infected with COVID-19, with booster doses where recommended and with the consensus to prioritise pregnant women if vaccines

are in limited supply [19]. As the majority of the reproductive-age population was vaccinated in the years 2021–2022, it is now imperative to seek a consensus on repeat boosters at the appropriate time during pregnancy, given the possible waning immunity and increased susceptibility to clinical infections in late pregnancy, a time associated with adverse perinatal outcomes. An alternative strategy may be to assess neutralizing activity during pregnancy to determine the relevance of an additional booster dose, particularly following a long interval between primary vaccination and pregnancy.

This study was conducted at a single academic hospital during a surveillance timeline that spanned the Delta- and Omicron-predominant periods. During the initial 2 years of the pandemic, global apprehensions regarding the potential adverse effects of the COVID-19 vaccine on fetal development and the possibility of long-term side effects extending beyond the neonatal phase were widespread. Consequently, there arose misconceptions and hesitancy toward vaccination among pregnant individuals, leading

to lower vaccination rates compared to the general population. These challenges were not only experienced globally but also within Singapore [20]. At the start of the study, most recruited women were unvaccinated; by the midway point, many women at recruitment had already received at least one dose pre-pregnancy or in very early gestation before confirmation of pregnancy. Thus, we recruited in a real-world manner as patients attended the antenatal clinics at our hospital, resulting in five distinct time points of primary vaccination. Our patient cohort is smaller than reported in other studies, which mainly focused on clinical outcomes only (hospital admission, clinical severity, neonatal antibody transfer), and almost all patients had mRNA vaccines as these were the first to be approved for use in pregnancy. Most women also had boosters, as pregnancy was regarded a risk factor for COVID-19-related complications, leading to clinical specialists and national guidelines recommending booster doses in pregnancy [21]. This stance has since been echoed in other opinions [22]. Pre-pregnancy vaccination without booster may have deferred infection to the second trimester, an observation likely related to waning immunity over 7 months falling within the antenatal period. In contrast, women vaccinated in T3 tended to have more protection against infection. Boosters taken later in pregnancy lengthened the disease-free interval. These subtle differences may be related to gestation-dependent changes in maternal immune cell antigen reactivity, which may influence responses to both vaccination and infection [23]. Increasing rates of COVID-19 infections close to term and delivery, as demonstrated in our cohort, have implications for maternal management and utilization of resources, such as negative pressure isolation facilities in settings where these are still used, and may result in maternal respiratory embarrassment, which was previously reported to occur more frequently in T3 with wild type SARS-CoV-2 infection. A mitigating factor may be a milder infection due to persistent Nab, as observed in our cohort, which may be managed outpatient as is the current practice in most countries. This should be a consideration for pregnant women following a long postvaccination period, particularly given the trend toward endemic COVID-19, where seasonal vaccinations may be beneficial [24].

The cPass kit is the first serological test approved by the FDA under emergency use authorization for the measurement of functional SARS-CoV-2 neutralizing antibodies [25]. Several studies have consistently shown that the in-vitro neutralizing titer may correlate well with vaccine efficacy and an individual's protection against symptomatic SARS-CoV-2 infection [26-28]. However, the implications of the presence of these Nab for individuals in the current COVID-19 endemic era with numerous variants that evade the host immune response remain unclear [24]. Durability and robustness of inhibitory activity, whether this confers protection against clinical infection by VOCs, and the need for repeated vaccinations remain the subject of ongoing research to determine the impact of Nab against COVID-19 [26]. We observed that the initially high inhibitory activity to wild-type virus was steadily lost over time, with the greatest attrition observed in uninfected mothers who received only two vaccinations. Women who received their booster dose or those who had COVID-19 infection (with or without a booster) were more likely to maintain baseline inhibitory levels of >90% without substantial waning, demonstrating the effectiveness of booster doses or hybrid immunity [29]. We are unable to discount the possibility of additional COVID-19 exposure and subclinical infection as factors explaining the maintenance of inhibition in some vaccinated mothers. The observed rise in Nab titers that increased from 3 to 5 months to >7 months may have been caused by exposure to new infections through close contacts or via subclinical or untested postvaccination infections, where this information was not provided or recorded. Inhibition of Omicron RBD may be helpful as recent surges in infection rate are

attributed to its subvariants [3]. Our data illustrates the possible role of high initial neutralizing activity to wild-type viruses as a marker for cross-reactivity to VOCs. The efficient neonatal Nab transfer observed by us and others is reassuring, as is the cross-protection to Delta and Omicron observed during vaccination at all stages of pregnancy. We did not monitor maternal Nab beyond the postpartum period, thus we cannot comment on the eventual loss, if any, of maternal protection. We were unable to collect consecutive neonatal blood samples, limiting our knowledge of sustained protection in the babies. We measured only Nab in sera, and we do not report binding antibody data (IgG, IgM), nor did we assess anti-spike protein antibodies, as we believe that the surrogate Nab titer is more widely used as an assessment of vaccine efficacy for licensure and clinical use. We also did not measure T-cell responses, which may further enhance protection.

There are some differences in our study compared to recent publications. We did not find associations between mode of delivery and vaccination [30], we provided both serological and clinical data over a longer time course [31], and reported durability, waning, and cross-reactivity to Delta and Omicron VOCs in pregnant women. Our data supports the observations in non-pregnant adults that a booster dose or COVID-19 infection reduces immune escape of Omicron VOC [32], which still causes significant adverse maternal effects despite existing immunity. Clinicians may deem it advisable to suggest additional doses during successive pregnancies to enhance protection against emerging VOCs, given the gradual waning of the initial humoral immunity over time. This precaution gains particular significance in anticipation of viral mutations that could result in increasingly sophisticated immune evasion mechanisms [32]. This viewpoint has been mirrored by other opinions [22]. Boosters in subsequent pregnancies can also be expected to maintain effective neonatal Nab transfer and provide cross-protection to novel variants. A recent Singaporean population-based cohort study of 7292 infants aged <6 months reported ~42% vaccine effectiveness in infants against Omicron sublineages, including XBB, and a lower risk of infection in infants attributed to maternal vaccination, which was further enhanced following a booster dose [15].

The MOH regards full vaccination as three doses of mRNA vaccine and offers a booster dose 1 year after the last dose to those at increased risk of severe COVID-19 [2,17]. Thus, ~23% of our cohort had booster doses as this became routine practice. At the time of our study, ~60% of the Singaporean population also had a primary COVID-19 infection [33] and an increasing number would have had second or third infections in the past year, with milder disease due to hybrid immunity [34]. Globally, however, complete primary vaccination consists of two vaccinations, and our data shows sustained high levels of Nab for at least 3 months and up to 7 months or more with this regimen. In low-resource communities with limited access to COVID-19 vaccines, this finding may be helpful to build management strategies around ensuring that reproductive-age women are fully vaccinated before pregnancy and possibly receive an antenatal booster to maintain protection. In conclusion, complete primary vaccination during any trimester may delay COVID-19 infection and maintain effective neutralizing activity throughout pregnancy, including robust cross-reactivity to Delta and Omicron variants and efficient maternal-neonatal transfer.

#### Author contributions

**AM, HLFA, RW, MC, CM:** conceived and designed the study; **LFW, SY:** provided technical expertise; **AM, HLFS, JZ, JPD, BPS:** performed data extraction; **HLFS, JZ, JPD, BPS, CM:** performed statistical analysis; **AM, MR, PD, AK, RL, BEY, PAT, DCL, LYAC, MC, CM:** contributed clinical interpretation; all authors were involved

in writing and editing the paper. All authors read and approved the final manuscript.

### Data sharing

The data generated during the current study are not publicly available due to ethical restrictions but are available from the corresponding author on reasonable request.

### Declarations of competing interest

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

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ijid.2024.107121](https://doi.org/10.1016/j.ijid.2024.107121).

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