



# SARS-CoV-2 mRNA vaccine induced higher antibody affinity and IgG titers against variants of concern in post-partum vs non-post-partum women

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## Summary

**Background** Limited knowledge exists in post-partum women regarding durability of SARS-CoV-2 vaccine-induced antibody responses and their neutralising ability against SARS-CoV-2 variants of concern (VOC).

**Methods** We elucidated longitudinal mRNA vaccination-induced antibody profiles of 13 post-partum and 13 non-post-partum women (control).

**Findings** The antibody neutralisation titres against SARS-CoV-2 WA-1 strain were comparable between post-partum and non-post-partum women and these levels were sustained up to four months post-second vaccination in both groups. However, neutralisation titers declined against several VOCs, including Beta and Delta. Higher antibody binding was observed against SARS-CoV-2 receptor-binding domain (RBD) mutants with key VOC amino acids when tested with post-second vaccination plasma from post-partum women compared with controls. Importantly, post-vaccination plasma antibody affinity against VOCs RBDs was significantly higher in post-partum women compared with controls.

**Interpretation** This study demonstrates that there is a differential vaccination-induced immune responses in post-partum women compared with non-post-partum women, which could help inform future vaccination strategies for these groups.

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**One Sentence Summary:** SARS-CoV-2 vaccine response in naïve vs. post-partum women.

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### Research in context

#### *Evidence before this study*

Earlier studies have demonstrated similar neutralising antibody response in post-partum women vs. non-partum women following SARS-CoV-2 vaccination. Antibody affinity maturation and durability of antibodies elicited by SARS-CoV-2 vaccination will play an important role in protection against emerging SARS-CoV-2 variants. However, limited knowledge exists regarding the impact of physiological changes induced by lactation on SARS-CoV-2 vaccination-induced antibody affinity maturation in post-partum vs control women and the durability of antibodies against circulating SARS-CoV-2 variants of concern.

#### *Added value of this study*

Longitudinal quantitative and qualitative analysis of the mRNA vaccine-induced antibody responses in lactating post-partum vs non-post-partum women was performed to determine antibody neutralisation titres across SARS-CoV-2 variants, as well as antibody binding, IgG binding and antibody affinity maturation to RBD's engineered to express key amino acid mutations in several VOCs. Importantly, higher antibody binding and affinity were observed against SARS-CoV-2 RBD mutants with post-second vaccination in plasma samples from post-partum women compared with controls.

#### *Implications of all the available evidence*

To define immune correlates of protection, future studies should investigate the durability of these high-affinity antibodies following SARS-CoV-2 vaccination in different populations against emerging SARS-CoV-2 variants that can provide protection against COVID-19.

## Introduction

The rapid emergence of SARS-CoV-2 VOCs around the globe<sup>1</sup> is associated with different levels of resistance to neutralisation by convalescent plasma, neutralising monoclonal antibodies, as well as post-vaccination sera.<sup>2–4</sup> During our study period in 2020 and the first half of 2021, circulating VOCs included the B.1.1.7 (Alpha), the B.1.351 (Beta), the P.1 (Gamma) and B.1.617.2 (Delta) strains.<sup>1,5–8</sup> As the pandemic continues, the list of VOC continues to evolve and grow with the recent emergence of fast-spreading highly transmissible Omicron variant.<sup>9</sup> Multiple studies are evaluating the effectiveness of SARS-CoV-2 vaccines against circulating SARS-CoV-2 strains and the emerging VOCs.<sup>10–14</sup> Interestingly, several VOCs share one or more common mutations in the RBD (i.e., N501, K417 and E484)<sup>1</sup> which may impact the ability of the host immune system to neutralise VOCs.<sup>11,15</sup>

During the SARS-CoV-2 vaccine evaluation, pregnant and lactating women were not included in some of

the large-scale clinical trials. More recently, it was shown that pregnant and lactating women benefit from SARS-CoV-2 vaccines and induce quantitatively equivalent humoral responses compared with non-pregnant women against the vaccine strain.<sup>16–18</sup> However, limited knowledge exists regarding the impact of physiological changes at post-partum stage on SARS-CoV-2 vaccination-induced antibody affinity maturation in post-partum vs non-post-partum women and the durability of antibodies against circulating SARS-CoV-2 variants of concern, as anti-SARS-CoV-2 antibody avidity correlates with clinical benefit to humans against COVID-19.<sup>19–22</sup>

In the current study, we performed longitudinal quantitative and qualitative analysis of the mRNA vaccine-induced antibody responses in lactating post-partum vs non-post-partum women to determine any potential differences in RBD-binding antibodies and effectiveness of neutralisation across SARS-CoV-2 variants. Both plasma and breast milk were collected before and after vaccination. Pseudovirus neutralization assay (PsVNA) was used to measure the vaccine-induced antibody neutralization titres against SARS-CoV-2 WA-1 strain and several VOCs. Surface plasmon resonance (SPR) was used to measure antibody binding, IgG binding and antibody affinity maturation to spike RBDs engineered to express key amino acid mutations of the SARS-CoV-2 VOCs following vaccination.

## Methods

### Study design

Lactating post-partum women and non-post-partum women were recruited by word-of-mouth at the University of Maryland School of Medicine. The study protocol was approved by the University of Maryland Institutional Review Board (IRB # HP-00092061 and HP-00094782). Written informed consent was obtained from each participant. Inclusion criteria for the lactating post-partum cohort were post-partum vaccination with a SARS-CoV-2 mRNA vaccine during lactation and continued lactation through the course of the study. Inclusion criteria for the non-post-partum cohort were female sex and vaccination with a SARS-CoV-2 mRNA vaccine. Race, underlying comorbidities, history of non-COVID-19 infections during sample collection period, immunomodulating medications were collected at the time of participant enrollment (Table S1). De-identified samples were obtained by the National Institutes of Health and U.S. Food and Drug Administration from participants enrolled at the University of Maryland School of Medicine studies with written informed consent (Table S2). Breastmilk samples were collected from mothers who supplied fresh breastmilk using mechanical breast pump expression on the morning of sample collection. Timing of samples in relation to infant

feeding was not standardized nor recorded. Once samples were collected, they were processed within 0–2 days of collection. Samples were heat inactivated and evaluated in different neutralisation and antibody-binding assays in duplicates in a blinded fashion.

### Pseudovirion neutralisation assay (PsVNA)

Antibody preparations were analyzed by SARS-CoV-2 pseudovirion neutralisation assay (PsVNA) using WA-1 strain, Alpha variant (B.1.1.7), Gamma variant (P.1 strain), Beta variant (B.1.351 strain) and Delta variant (B.1.617.2 strain), as described previously.<sup>19,23,24</sup>

Briefly, human codon-optimized cDNA encoding SARS-CoV-2 S glycoprotein of the WA-1/2020 and variant strains (Table S3) were synthesized by GenScript and cloned into eukaryotic cell expression vector pcDNA 3.1 between the *Bam*HI and *Xho*I sites. Pseudovirions were produced by co-transfection of Lenti-X 293T cells with psPAX2(gag/pol), pTrip-luc lentiviral vector and pcDNA 3.1 SARS-CoV-2-spike-deltaC19 using Lipofectamine 3000. The supernatants were harvested at 48 h post transfection and filtered through 0.45 µm membranes and titrated using 293T-ACE2-TMPRSS2 cells (HEK 293T cells that express ACE2 and TMPRSS2 proteins). The cell lines used in this study were tested and validated as described previously.<sup>25</sup> Mycoplasma testing was not performed on the cell lines.

For the neutralisation assay, 50 µL of SARS-CoV-2 S pseudovirions were pre-incubated with an equal volume of medium containing samples at 3-fold serial dilutions (starting plasma dilution of 1:10 or 1:5 for milk) at room temperature for 1 h. Virus-antibody mixtures were subsequently added to 293T-ACE2-TMPRSS2 cells<sup>25</sup> in a 96-well plate. The input virus with all eight SARS-CoV-2 strains used in the current study were the same ( $2 \times 10^5$  Relative light units/50 µL/well). After a 3 h incubation, fresh medium was added to the wells. Cells were lysed 24 h later, and luciferase activity was measured using luciferin. Controls included cells only, virus without any antibody, and positive sera. The cut-off value or the limit of detection for the neutralisation assay is 1:20. Seropositivity was defined as PsVNA<sub>50</sub> neutralisation titers of  $\geq 60$ .

### Proteins

Recombinant SARS-CoV-2 spike RBD and its mutants were purchased from Sino Biologicals (RBD; 40592-V08H, RBD-K417N; 40592-V08H59, RBD-N501Y; 40592-V08H82 and RBD-E484K; 40592-V08H84). Recombinant purified RBD proteins used in the study were produced in 293 mammalian cells. The native receptor-binding activity of the spike RBD proteins was determined by binding to 5 µg/mL of human ACE2 protein.<sup>19,21,23</sup>

### Antibody binding kinetics of post-SARS-CoV-2 vaccination human samples to recombinant SARS-CoV-2 RBD proteins by SPR

Steady-state equilibrium binding of post-SARS-CoV-2 infected human polyclonal sample was monitored at 25 °C using a ProteOn surface plasmon resonance (BioRad). The purified recombinant SARS-CoV-2 proteins were captured to a Ni-NTA sensor chip with 200 resonance units (RU) in the test flow channels. The protein density on the chip was optimized such as to measure monovalent interactions independent of the antibody isotype.<sup>26</sup> Serial dilutions (10-, 50- and 250-fold) of freshly prepared sample in BSA-PBST buffer (PBS pH 7.4 buffer with Tween-20 and BSA) were injected at a flow rate of 50 µl/min (120 s contact duration) for association, and disassociation was performed over a 600 s interval. Responses from the protein surface were corrected for the response from a mock surface and for responses from a buffer-only injection. Total antibody binding was calculated with BioRad ProteOn manager software (version 3.1). All SPR experiments were performed twice. In these optimized SPR conditions, the variation for each sample in duplicate SPR runs was <6%. The maximum resonance units (Max RU) shown in figures is for 10-fold diluted plasma/milk sample.

Antibody off-rate constants, which describe the stability of the antigen-antibody complex (i.e. the fraction of complexes that decays per second in the dissociation phase) were determined directly from the interaction of human polyclonal samples with recombinant purified SARS-CoV-2 RBD proteins using SPR in the dissociation phase only for the sensorgrams with Max RU in the range of 10–150 RU and calculated using the BioRad ProteOn manager software for the heterogeneous sample model as described before.<sup>23,26,27</sup> Off-rate constants were determined from two independent SPR runs.

SARS-CoV-2 RBD-specific IgG was quantified by capturing 10-fold dilution of plasma collected at day 14 following first or second vaccination on anti-human IgG-Fc sensor chips, followed by injection of WA-1 RBD or RBD mutant proteins for 120 s for association. Responses from the protein surface were corrected for the response from a mock surface and for responses from a buffer-only injection. Total antibody binding was calculated with BioRad ProteOn manager software (version 3.1).

### Statistical analysis

All experimental data to compare differences between groups were analyzed using lme4 and emmeans packages in R (RStudio version 1.1.463).

The initial baseline demographics of these patients are shown in Tables S1 and S2. A parametric *t*-test was performed to compare age between the post-partum and non-post-partum groups. A non-parametric *t*-test

was performed between the post-partum and non-post-partum groups for all other demographics (Table S2).

Since age can be a biologically plausible confounder, data from SPR (antibody binding and antibody off-rates) and neutralisation titers (absolute values) were analyzed for statistical significance amongst post-partum vs non-post-partum women to control for age as covariate (predictor variables) using a linear regression model. To ensure robustness of the results, absolute measurements were log<sub>2</sub>-transformed before performing the analysis. For comparisons between two vaccine categories (factor variable), pairwise comparisons were extracted using 'emmeans' and Tukey-adjusted *p* values were used for denoting significance to reduce Type I error due to multiple testing. The tests were two-sided tests.

Experiments were performed based on sample availability during the vaccine study and hence sample size calculations were not done a priori. Power analysis for sample size calculations were performed assuming a power value (beta) as 0.95, 0.9 and 0.8, in the order of decreasing stringency to eliminate Type I error. A significance level of 0.05 was used for sample size calculations. These calculations showed that we needed a sample size of 13, 11 and 8, respectively that are within the actual sample size used in the current study. Hedge's 'g' was determined for effect size calculations using the 'effsize' package in R.

Samples were allocated randomly to each test group and tested in blinded fashion (researcher was blinded to sample identity) to minimize selection bias or detection bias. There were no exclusion criteria. All samples and data were used for analysis and presented in the study.

#### Data and materials availability

All data are present in the manuscript and/or the Supplementary Materials. The materials generated during the current study are available from the corresponding author under a material transfer agreement on reasonable request.

**Ethics statement.** The study at CBER, FDA, was conducted with de-identified samples and all assays performed fell within the permissible usages in the original consent. Antibody assays were performed with approval from the U.S. Food and Drug Administration's Research Involving Human Subjects Committee (FDA-RIHSC) under exemption protocol '252-Determination-CBER-2020-08-19.

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## Results

### Patient cohort demographics

Thirteen lactating post-partum women and 13 non-post-partum control women were compared to observe differences in vaccine efficacy. The lactating cohort consisted of 92.3% White and 7.7% Asian with an average age of 34 years (Table S2). The non-post-partum women were 84.6% White and 15.4% African American with an average age of 41.5 years. There was no statistically significant difference between the races ( $p = 0.4800$ ) or ages ( $p = 0.0743$ ) in the two cohorts. Twenty-three percent of lactating women and 35% of non-post-partum women received the BNT162b2 mRNA vaccine ( $p > 0.999$ ). The average BMI of lactating women was 25 and non-post-partum women was 21.7 ( $p = 0.2393$ ). Three lactating women reported pulmonary-related comorbidities, and one reported a non-pulmonary-related comorbidity. One non-post-partum female reported a pulmonary-related comorbidity, and one non-post-partum female reported a non-pulmonary-related comorbidity. There was no difference in the numbers of reported comorbidities in the two cohorts ( $p = 0.584$ ); pulmonary ( $p > 0.999$ ) or non-pulmonary ( $p = 0.5179$ ). Ten lactating women and thirteen non-post-partum women reported symptoms after first vaccination. Twelve lactating women and thirteen non-post-partum women reported symptoms after a second vaccination. Two lactating women reported illness after vaccination. There was no difference between the numbers of participants in each cohort with reported symptoms after first vaccination ( $p = 0.2200$ ) symptoms after second vaccination ( $p > 0.999$ ), or illness after vaccination ( $p > 0.999$ ).

### Neutralising antibody titers of post-vaccination plasma and breast milk against SARS-CoV-2 variant strains

The objective of this longitudinal study was to investigate the post-vaccination induced quantitative and qualitative antibody responses in lactating women ( $N = 13$ ) compared with non-post-partum control women ( $N = 13$ ) against vaccine-homologous SARS-CoV-2 strain (WA-1) and several emerging VOCs including Alpha (B.1.1.7), Gamma (P.1), Beta (B.1.351) and Delta (B.1.617.2) strains (Table S2). None of the 26 female participants reported any prior SARS-CoV-2 infection

and were confirmed seronegative by testing of plasma samples collected prior to vaccination ( $D = 0$ ) by ELISA using SARS-CoV-2 spike and nucleocapsid proteins. All subjects received two doses of mRNA vaccine either from Moderna (mRNA-1273) or from Pfizer-BioNTech (BNT162b2) at 4-week or 3-week intervals between doses, respectively (Figure 1a,b).

The baseline demographics of these study participants did not result in any significant differences between the two groups of women, suggesting that the two populations are similar and appropriate for antibody comparisons in this study (Table S1).

Plasma samples were collected two weeks after each of the first and second vaccination doses in the series as well as 3-months after completing the vaccine series. Morning breast milk samples were collected from post-partum women following vaccination, corresponding to the day of plasma collection. There was no difference in the quantitative or qualitative antibody responses between the vaccine types. Therefore, all data analyses were conducted irrespective of vaccine type.

The Pseudovirus neutralisation assay (PsVNA) was performed using 293-ACE2-TMPRSS2 cell line as previously described.<sup>19,23</sup> A PsVNA<sub>50</sub> titer above 1:60 was used as a seropositive cut-off based on current understanding of neutralising antibody as correlate of protection against COVID-19.<sup>28</sup> The pre-vaccination PsVNA<sub>50</sub> titers against vaccine homologous SARS-CoV-2 WA-1 were <1:20 for all individuals (limit of detection) (Figure 1c). Following the first vaccination, 6 of 13 control and 8 of 13 post-partum women showed a vaccine-induced response with plasma PsVNA<sub>50</sub> titers >1:60, with mean titers of 1:409 and 1:136, respectively (Figure 1c,d). In addition to vaccine-homologous WA-1 strain, we measured virus-neutralising antibody titers against SARS-CoV-2 VOCs (Figure S1). Neutralising antibodies against VOCs after the first vaccination were lower compared with WA-1 antibodies at the same time-point, with 2.3 to 11-fold reduction for the non-post-partum control group and between 1.4 and 7.1-fold reduction for the post-partum group compared with WA-1 strain (Figs. 1f–h and S1). There were no significant differences between the control and post-partum group in post-first vaccination PsVNA<sub>50</sub> titers against either WA-1 ( $p = 0.825$ ) or any of the four VOCs tested ( $p = 0.247$  to  $0.603$ ) (Figure 1h).

The second mRNA vaccination boosted the plasma immune response with high WA-1 neutralising titers at 2-weeks post-second vaccination in both non-post-partum controls and post-partum women (mean PsVNA<sub>50</sub> of 1:2608 and 1:3487, respectively) with 100% seropositivity (Figure 1c,d). In breast milk of post-partum women, the PsVNA<sub>50</sub> titers after the first vaccination were negative (<1:60). After the second vaccine dose, an increase was observed (mean PsVNA<sub>50</sub> of 1:72) in neutralising antibodies, with one post-partum female showing milk PsVNA<sub>50</sub> titers of 1:645 against WA-1 at day 14 post vaccination (Figure 1e).

The post-second vaccination plasma of post-partum women were 2 to 3-fold higher neutralisation titers against VOC compared with plasma from non-post-partum controls (Figure 1f,g). However, this difference failed to reach statistical significance ( $p = 0.115$  to  $0.948$ ) (Figure 1i). Among the controls, significant reduction in post-second PsVNA<sub>50</sub> titers were observed against B.1.351 (13-fold;  $p = 0.0016$ ), followed by B.1.617.2 (7.4-fold;  $p = 0.0013$ ) and P.1 (6.8-fold;  $p = 0.0025$ ). In post-partum women, the reduction in neutralisation was less but remained significant against B.1.351 (7.5-fold;  $p = 0.019$ ), B.1.617.2 (5-fold;  $p = 0.038$ ) and P.1 (3.2-fold;  $p = 0.031$ ), compared with WA-1 strain (Figure 1f,g). There was no significant reduction in B.1.1.7 titers compared with WA-1 in either group. Seropositivity against SARS-CoV-2 VOCs ranged between 82 and 100% with lowest rates against B.1.351 VOC (Figure 1i). Weak neutralising antibody response was observed in breast milk against the VOCs (Figure S1).

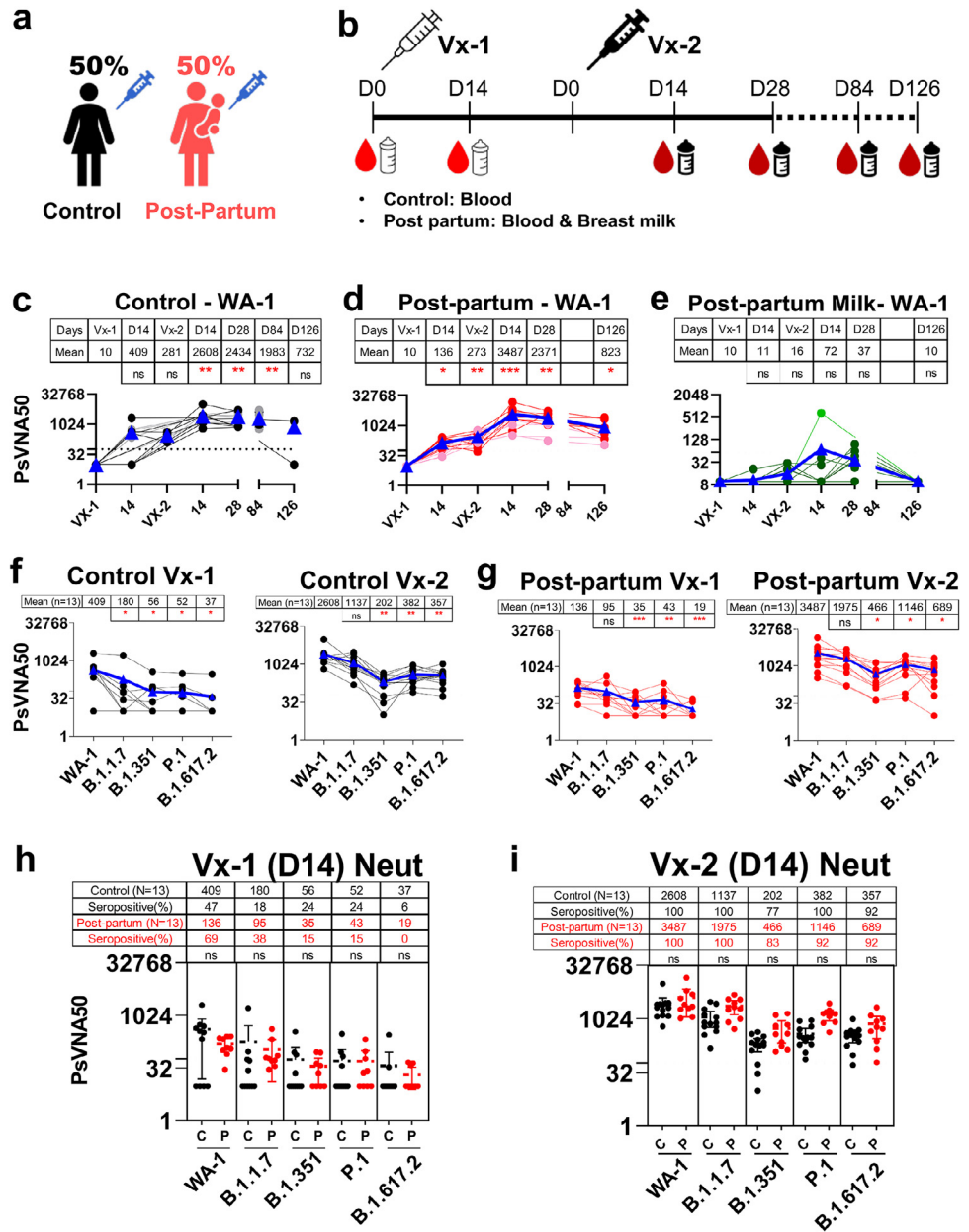
By 4 months post-second vaccination, the plasma PsVNA<sub>50</sub> titers to WA-1 and VOCs declined in both groups (Figs. 1c, d and S1). While majority of samples were still seropositive (titer >1:60) against WA-1, ~30% of participants were seronegative against the B.1.351 and B.1.617.2 VOC (Figure S1), by 4 months post-second vaccination.

### Vaccination induced binding antibodies against SARS-CoV-2 RBD and its mutants

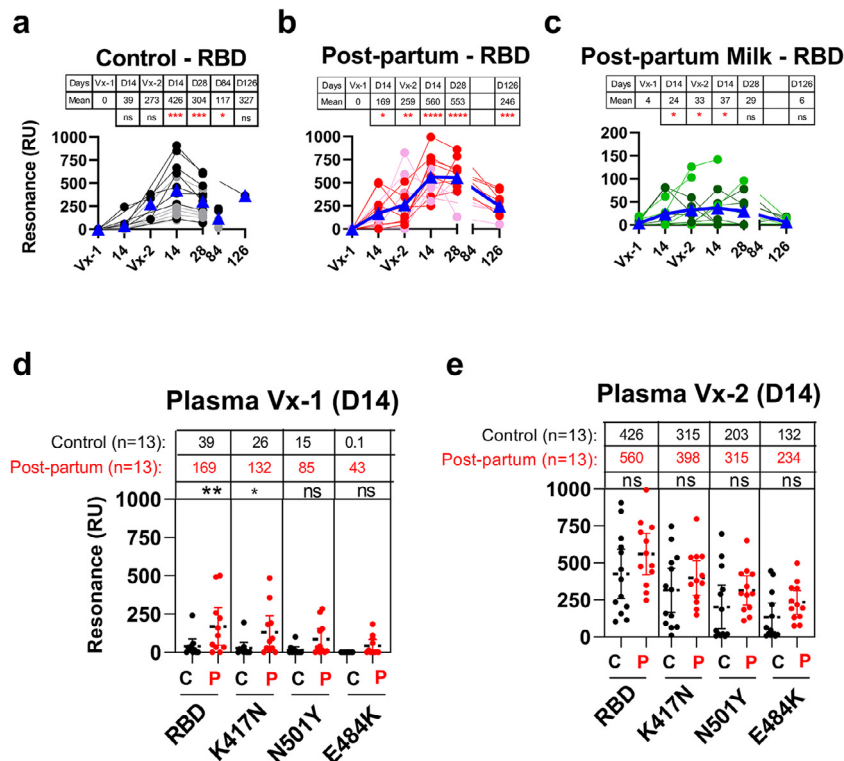
Steady-state equilibrium binding of longitudinally collected vaccinated plasma from non-post-partum women (control) vs plasma and breast milk from post-partum women was monitored using SPR against RBD of WA-1 (RBD) and RBD proteins containing key amino acid mutations K417N (found in B.1.351), N501Y (found in B.1.1.7, B.1.351 and P.1) and E484K (found in B.1.351, P.1, and B.1.617.1) (Table S3). None of the individuals demonstrated the presence of RBD-binding antibodies at pre-vaccination baseline.

Following the first mRNA vaccination, the antibody binding (Max RU) to WA-1 RBD increased in both groups. This effect was further boosted by second vaccine dose with the same mRNA vaccine as the first dose in all individuals (Figure 2a,b). The RBD-binding antibodies in milk of post-partum women increased 6-fold after the first vaccine dose and were marginally boosted by a second vaccine dose but declined to baseline levels at 4 months post-vaccination (Figure 2c).

Vaccination induced binding antibodies to the RBD single mutants (K417N, N501Y and E484K) (Figure S2a). However, antibody binding to the mutants was significantly lower than binding to the vaccine-homologous WA-1 RBD at two weeks (D14) after either the first or the second vaccination in both groups. The lowest antibody binding was observed to the RBD-E484K



**Figure 1.** Neutralising antibody titers of post-vaccination plasma or breast milk in post-partum women and non-post-partum women against various SARS-CoV-2 strains. (a) Overview of vaccination cohort, including non-post-partum women (Control;  $n = 13$ ) and post-partum women ( $n = 13$ ). (b) Timeline of SARS-CoV-2 vaccination and sample collection in the two female cohorts. (c-i) SARS-CoV-2 neutralising antibody titers in plasma of 13 non-post-partum (black) vs. plasma (red) or milk (green) of 13 post-partum women as determined by pseudovirus neutralisation assay (PsVNA) in 293-ACE2-TMPRSS2 cells with SARS-CoV-2 WA-1 strain, B.1.1.7 variant, P.1 variant, B.1.351 variant or B.1.617.2 variant. PsVNA50 (50% neutralisation titer) titers of pre-vaccination (D0 of Vx-1), post-1st (D14) or at different time-points following second vaccination (Vx-2) plasma samples for Controls (c) or plasma (d) and breast milk (e) from post-partum women against the vaccine-matched WA-1 strain. Mean PsVNA50 titers values are shown as blue triangles and are presented for each vaccination time-point against the SARS-CoV-2 WA-1 on top of the panel. (f-g) Plots showing mean values  $\pm$  range of PsVNA50 neutralisation titers with 2-weeks post-first vaccination plasma and post-second vaccination of non-post-partum controls (f) or post-partum women (g). (h,i) Comparisons of PsVNA50 (50% neutralisation) titers against WA-1 and B.1.1.7, P.1, B.1.351 and B.1.617.2 VOCs for post-first (h) and post-second mRNA vaccinated (i) plasma from non-post-partum controls ( $n = 13$ ; in black) or post-partum women ( $n = 13$ ; in red). The numbers above the group shows the mean for each variant are color coded for each of the group matching the colors in the graph. The horizontal dashed line indicates the seropositive cut-off of



**Figure 2.** Binding antibodies in post-mRNA vaccination plasma or breast milk in post-partum female's vs non-post-partum women against vaccine-homologous SARS-CoV-2 receptor binding domain and its mutants. (a-c) Total antibody binding (determined by maximum resonance units, Max RU) of 1:10 diluted pre-vaccination (D0 of Vx-1), post-1st (D14) or at different time-points following second vaccination (Vx-2) plasma samples of 13 non-post-partum (a; black) vs. plasma (b; red) or breast milk (c; green) of 13 post-partum women to purified WA-1 RBD (RBD) and RBD mutants: RBD-K417N, RBD-N501Y and RBD-E484K by SPR. Mean antibody binding values are shown as blue triangles and are presented for each RBD. (d,e) Mean values  $\pm$  range of total antibody binding (Max RU) of 10-fold diluted post-1st (d) or post-2nd (e) vaccination sample from non-post-partum controls ( $n = 13$ ; in black) or post-partum women ( $n = 13$ ; in red), against purified WA-1 RBD (RBD) and RBD mutants: RBD-K417N, RBD-N501Y and RBD-E484K by SPR. The mean values for Max RU for each RBD are color coded by each group. All SPR experiments were performed in duplicate, and the researchers performing the assay were blinded to sample identity. The variations for duplicate runs of SPR were  $<5\%$ . The data shown are average values of two experimental runs. The statistical significances between the variants were performed using *R* that controlled for age as a covariate. The differences were considered statistically significant with a 95% confidence interval when the *p* value was less than 0.05. (\* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ ).

mutation compared with WA-1 RBD (Figure S2b). The mean post-first vaccination plasma antibody binding to RBD or its mutants for the post-partum group was 4.3 to 43-fold higher than that of the control group, with a significant difference in the control and post-partum group for RBD ( $p = 0.0047$ ) and RBD-K417N ( $p = 0.039$ ) (Figure 2d). The difference in post-second RBD-binding titers was only 1.3 to 1.8-fold and was not significant between these groups (Figure 2e).

#### Durability of SARS-CoV-2 neutralisation and RBD-binding antibodies following SARS-CoV-2 mRNA vaccination

To determine if post-partum physiological factors may influence the longevity of vaccination-induced immune response against SARS-CoV-2, we evaluated the durability of antibody response of post-partum women vs. non-post-partum female controls up to 4 months post-vaccination. The PsVNA<sub>50</sub> titers against WA-1 and the four

PsvNA50 titer of 1:60. Seropositivity was defined as PsVNA50 neutralisation titers of  $>60$ . All PsVNA experiments were performed in duplicate and the researchers performing the assay were blinded to sample identity. The variations for duplicate runs were  $<6\%$ . The data shown are average values of two experimental runs. The statistical significances between the variants within each cohort were performed using *R* and controlled for age as a covariate. The differences were considered statistically significant with a 95% confidence interval when the *p* value was less than 0.05. (\* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ ).

SARS-CoV-2 variants declined over time but remained above the neutralisation titers observed for post-first vaccination against all strains (Figs. 1c,d and S1). While failing to reach statistical significance, there was a trend towards higher neutralisation titers in post-partum women against B.1.1.7 and B.1.351 compared with non-post-partum controls (Figure S1).

The decline in post-second vaccination binding antibodies to RBD and its mutants at the 3 to 4-months' time-point was ~2-fold from their peak binding titers (on day 14 post-second vaccination) and was similar for both the groups (Figs. 2a,b and S2a). No significant differences were observed for RBD-binding antibodies between the two groups.

#### Vaccination induced antibody affinity maturation in plasma of post-partum female's vs non-post-partum women to SARS-CoV-2 RBD

Physiological changes induced by lactation may influence the antibody affinity of vaccination-induced antibody response against SARS-CoV-2 in post-partum women vs. non-post-partum women. As a surrogate of antibody affinity, antibody off-rate constants, which reflect the stability of the antigen-antibody complex, were determined directly from (serially-diluted human polyclonal) plasma sample interaction with SARS-CoV-2 RBD and its mutants using SPR in the dissociation phase only for the sensorgrams with Max RU in the range of 10–150 RU, as described before.<sup>23,26,27</sup>

For post-partum women, the dissociation rates of the post-first-vaccination plasma antibodies against vaccine-homologous SARS-CoV-2 WA-1 RBD were fast (i.e., low antibody affinity; ranging between 0.1 and 0.01 per s), but non-post-partum controls showed even faster (~2-fold) dissociation rates (Figure 3a). Post-first vaccination antibody affinity against RBD-mutants was lower (faster dissociation rate) for both groups. None of the plasma samples from non-post-partum controls following vaccination showed binding to RBD-E484K higher than 10 RU, therefore, antibody affinity could not be determined against this RBD mutant for the control group.

After the second vaccination, the affinity of the antibodies increased, demonstrating slower dissociation in both groups at peak antibody response on day 14 (Figure 3b). Surprisingly, the post-second vaccination plasma antibody affinity was significantly higher (lower antibody off-rates;  $p = 0.023$  to  $0.0034$ ) for post-partum women compared with non-post-partum controls against all RBD proteins. Following the second vaccination, antibody affinities against WA-1 RBD in non-post-partum women were 2.3-fold lower compared with post-partum women (Figure 3b). This difference in antibody off rates was reduced, but still statistically significant, when comparing the binding to RBD-K417N ( $p = 0.023$ ) and RBD-N501Y ( $p = 0.012$ ) at 2-weeks post-second dose of the two groups. Post-second vaccination plasma from

post-partum women demonstrated 2.5-fold higher antibody affinity (slower off-rates;  $p = 0.0034$ ) against the RBD-E484K compared with plasma from non-post-partum women (Figure 3b).

Surface plasmon resonance measurement determines contribution of all antibody isotype (IgG, IgM, and IgA) binding to RBD. To elucidate the difference in antibody affinity observed between the two groups, we next probed the titer of IgG specific for SARS-CoV-2 RBD and its mutants at 2-weeks (peak titers) post-first and post-second vaccination plasma from the two groups. These analyses revealed 9 to 13-fold significantly ( $p = 0.0009$  to  $0.0004$ ) higher SARS-CoV-2 RBD-binding IgG after the first vaccination (Figure 3c) and 4 to 5-fold higher RBD-binding IgG ( $p = 0.0008$  to  $0.0002$ ) after the second vaccination (Figure 3d) in post-partum women compared with non-post-partum control against vaccine-homologous RBD and RBD single-point mutants.

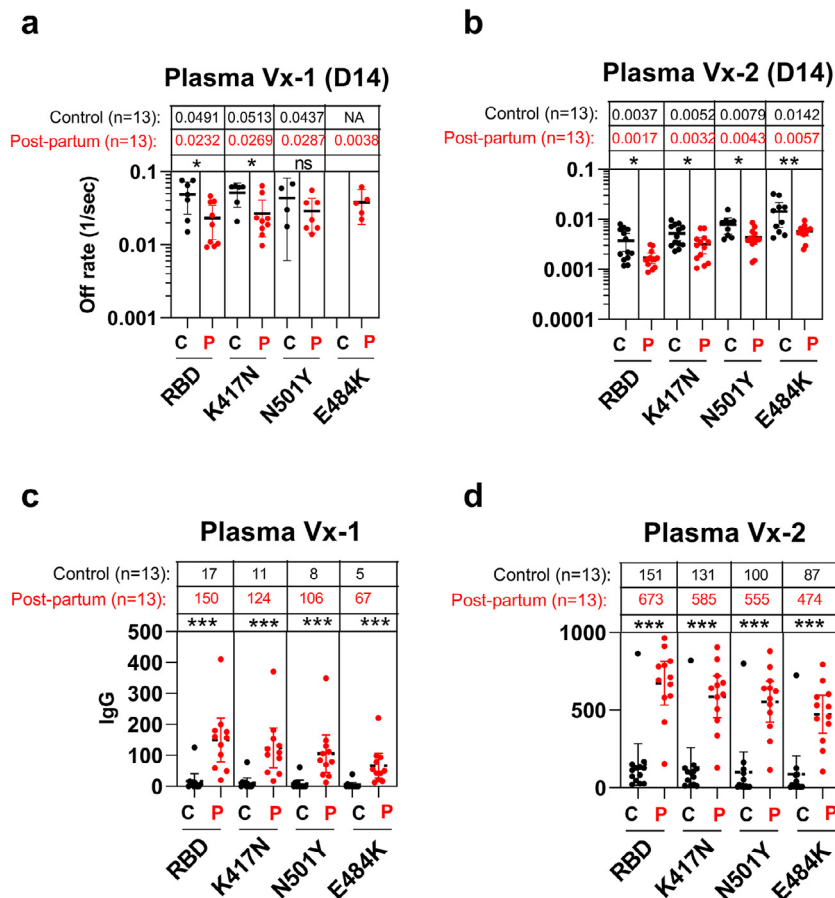
These data indicated that the composition of SARS-CoV-2 mRNA vaccine-induced antibodies in post-partum women was different from non-post-partum women. Vaccination of post-partum women generated higher IgG titers and stronger antibody affinity to spike RBD of SARS-CoV-2 VOCs/VOIs than non-post-partum women.

#### Discussion

Our study revealed quantitative and qualitative differences in the antibody responses of post-partum women compared with non-post-partum women following mRNA vaccination. In these groups, cross neutralisation of VOCs was minimally reduced against the B.1.1.7 (Alpha) but dropped substantially against the P.1 (Gamma), B.1.351 (Beta) and B.1.617.2 (Delta). However, the post-second vaccination peak neutralisation responses and durability at 4 months against SARS-CoV-2 as well as Beta and Gamma VOCs were 2-fold higher in post-partum participants compared with the non-post-partum vaccine recipients (Figure 1c,d and i).

We observed low SARS-CoV-2 neutralising and RBD-binding antibodies in human milk after vaccination in agreement with prior studies.<sup>17,18</sup> However, the antibody transfer to infants and their importance in protection against SARS-CoV-2 through breastfeeding remains to be established. The protective efficacy by vaccine induced antibodies against emerging variants may be impacted by both specific amino acid mutations in the RBD and specificity/affinity of the polyclonal antibodies that bind to SARS-CoV-2 spike. We also observed significantly higher affinity of antibodies to the RBD and its mutants in post-partum women compared with non-post-partum controls after second vaccination, a phenomenon that has not been described before, and was not predicted by the neutralisation titers measured in these participants. Pregnancy is a state of immune





**Figure 3.** Antibody affinity maturation of human antibody response following SARS-CoV-2 mRNA vaccination in non-post-partum vs post-partum women. (a-b) Polyclonal antibody affinity maturation (as measured by dissociation off-rate per seconds) to SARS-CoV-2 RBD proteins for post-first (Vx-1; a) or post-second vaccination (Vx-2; b) plasma samples at peak titers (day 14) following each vaccination for non-post-partum controls ( $n = 13$ ; in black) or post-partum women ( $n = 13$ ; in red), was determined by SPR. Antibody off-rate constants that describe the fraction of antibody-antigen complexes decaying per second were determined directly from the serially diluted post-vaccination sample interaction with SARS-CoV-2 RBD proteins using SPR in the dissociation phase as described in Materials and Methods. Off-rate was calculated and shown only for the sample time points that demonstrated a measurable ( $>10$ RU) antibody binding in SPR. Antibody affinity of post-first vaccination plasma from non-post-partum women against RBD-E484K (a) were not determined since the RBD binding antibodies were  $<10$ RU for these samples. (c,d) RBD-specific IgG against SARS-CoV-2 RBD and its mutants for the post-first (c) and post-second (d) vaccination plasma from non-post-partum controls ( $n = 13$ ; in black) or post-partum women ( $n = 13$ ; in red). The mean values are color coded by each group. All SPR experiments were performed twice and the researchers performing the assay were blinded to sample identity. The variation for each sample in duplicate SPR runs was  $<5\%$ . The data shown are the average value of two experimental runs. The statistical significances between the groups or different time-point samples were performed using  $R$  that controlled for age as covariate. The differences were considered statistically significant with a 95% confidence interval when the  $p$  value was less than 0.05. (\* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ ).

alteration allowing woman and fetus to live in harmony. It is predominated by Th<sub>2</sub> responses that quickly shift to Th<sub>1</sub>-dominant responses post-partum, and immune reconstitution post-partum might contribute to hyperinflammatory responses to infection.<sup>29,30</sup> During pregnancy, women can mount and transfer robust immune responses to their infants, therefore, several maternal vaccinations are recommended against infectious diseases. To understand the difference in antibody affinity we also evaluated the RBD-specific IgG in the vaccine

response of post-partum and non-post-partum women. Significantly higher RBD-specific IgG were observed in post-partum women compared with non-post-partum women, suggesting that antibody class-switching to IgG was the primary drive for the observed higher-antibody affinity in post-partum women. It's possible that physiological as well as immune reconstitution following pregnancy in post-partum women may drive overall activation of the immune system or germinal center formation resulting in "more robust" antibody-class

switching and antibody affinity maturation following vaccination. However the exact mechanism requires further investigation.

In previous studies, we had demonstrated a strong correlation between antibody affinity and protection from challenge with highly pathogenic avian influenza viruses in the ferret model<sup>31,32</sup> and a correlation with lower disease scores and clinical benefit in patients infected with Zika virus,<sup>33</sup> Ebola virus,<sup>26</sup> influenza virus.<sup>34</sup> We previously observed increased antibody affinity maturation without significant increase in SARS-CoV-2 neutralisation or antibody binding in adults diagnosed with COVID-19 and children with COVID-19 and MIS-C, and identified antibody affinity was as immune correlate of disease resolution.<sup>19–22</sup> Therefore, in addition to virus neutralisation, it is important to measure antibody affinity maturation against the entire SARS-CoV-2 spike in order to fully capture the evolution of antibodies after first and second vaccination. Affinity maturation may influence the protective efficacy of vaccines in individuals especially against current VOCs and emerging new variants.

One of the limitations of this study is the small sample size. This was a single center study of patients derived from a convenience sampling. The diversity of the cohorts was limited by recruitment through word of mouth. As such, the outcomes of this study are exploratory and hypothesis generating and merit further evaluation in a large cohort to confirm the reported findings. Other limitations of the interpretation of the breast milk data are that timing of sample acquisition with respect to infant feeding was not specified, and it is unknown if samples collected were fore or hind milk.

This study suggests a differential antibody response in post-partum vs non-post-partum women following SARS-CoV-2 vaccination. Therefore, future studies should investigate the potential population-specific differences in antibody response especially antibody affinity maturation and durability of high-affinity antibodies following SARS-CoV-2 vaccination and the correlation between antibody affinity and vaccine efficacy against emerging SARS-CoV-2 variants that can provide protection against COVID-19.

#### Contributors

All authors read and approved the final version of the manuscript.

#### Designed research

S.K.

#### Performed research

Y.L., J.T., G.G., S.R. and S.K. G. G. and S.K. verified the underlying data.

#### Collected clinical samples and provided clinical data

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#### Contributed to writing

S.K.

#### Data sharing

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

#### Declaration of interests

The authors have nothing to declare.

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#### Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.ebiom.2022.103940](https://doi.org/10.1016/j.ebiom.2022.103940).

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