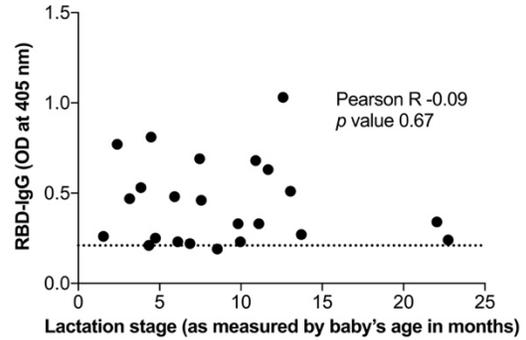
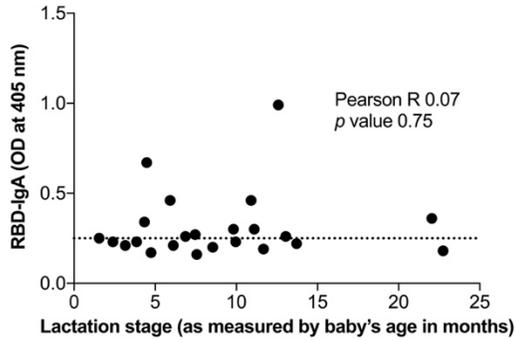
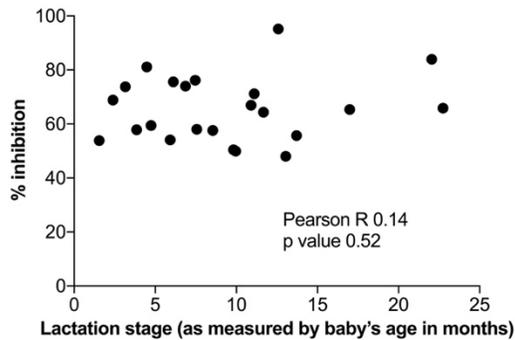


**Appendix 2. No relationship between lactation stage and immune response in milk. A–B. Correlation between milk anti-receptor-binding domain (RBD) antibodies and lactation stage as measured by baby’s age (months). C. Correlation between milk neutralization of wildtype spike and lactation stage as measured by baby’s age (months). Pearson’s correlation coefficient (R) and *P*-values are shown.**

**A** Correlation between milk anti-RBD IgA and baby’s age      **B** Correlation between milk anti-RBD IgG and baby’s age



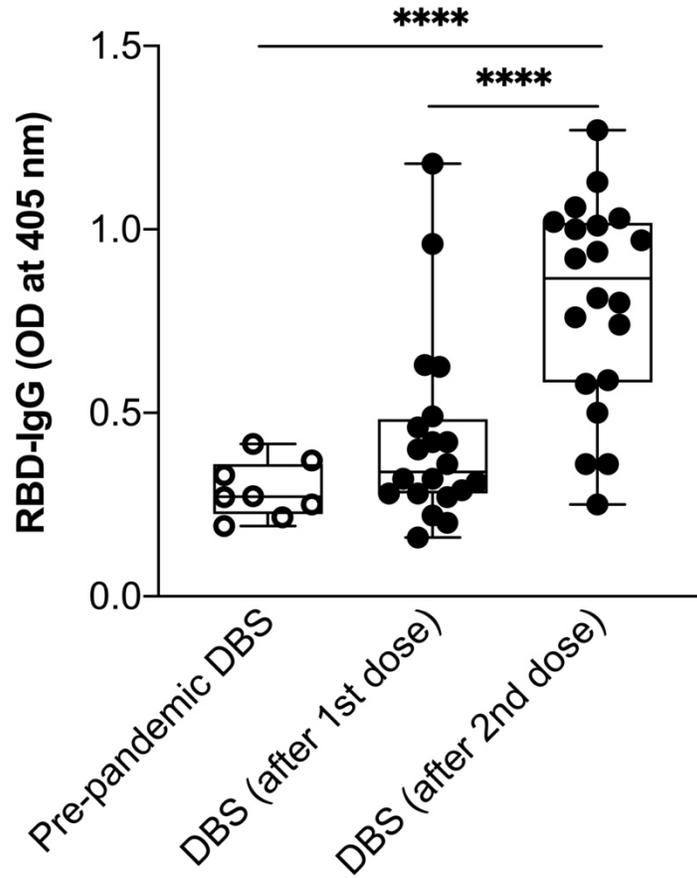
**C** Correlation between milk neutralization ability and baby's age



Narayanaswamy V, Pentecost B, Schoen CN, Alfandari D, Schneider SS, Baker R, et al. Neutralizing antibodies and cytokines in breast milk after coronavirus disease 2019 (COVID-19) mRNA vaccination. *Obstet Gynecol* 2022;139. The authors provided this information as a supplement to their article.

**Appendix 3. Anti-receptor-binding domain (RBD) immunoglobulin (Ig)G detected in dried blood spots (DBS) 21 days after the second dose of vaccination. Comparison of anti-RBD IgG in DBS eluates provided by women in the pre-pandemic control group (n=8), by women 19 days after the first vaccine dose and 21 days after the second dose (n=21). Differences between DBS first dose and DBS second dose were assessed with a matched paired *t*-test. Differences between DBS second dose and prepandemic DBS controls were assessed with an independent *t*-test. \*\*\*\* $P \leq .0001$ .**

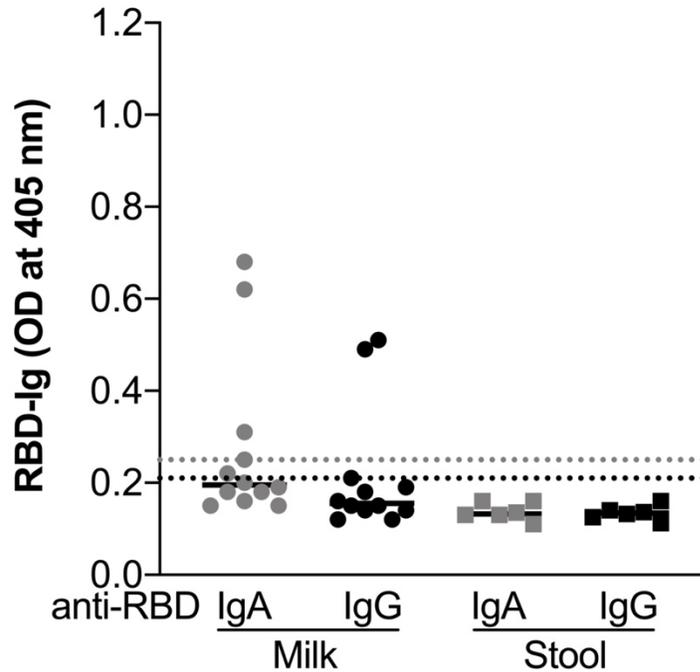
### Dried blood spot cards



Narayanaswamy V, Pentecost B, Schoen CN, Alfandari D, Schneider SS, Baker R, et al. Neutralizing antibodies and cytokines in breast milk after coronavirus disease 2019 (COVID-19) mRNA vaccination. *Obstet Gynecol* 2022;139. The authors provided this information as a supplement to their article.

**Appendix 4. Anti-receptor-binding domain (RBD) immunoglobulin (Ig)A and IgG are not detected in pre-pandemic milk and stool controls. Levels of milk anti-RBD IgA and IgG (circle; n=12) and levels of infant stool anti-RBD IgA and IgG (square; n=6) detected by enzyme-linked immunosorbent assay (ELISA). *Dotted lines* indicate positive cut-off values for anti-RBD IgA (*grey*) and anti-RBD IgG (*black*). *Solid lines* indicate median ODs.**

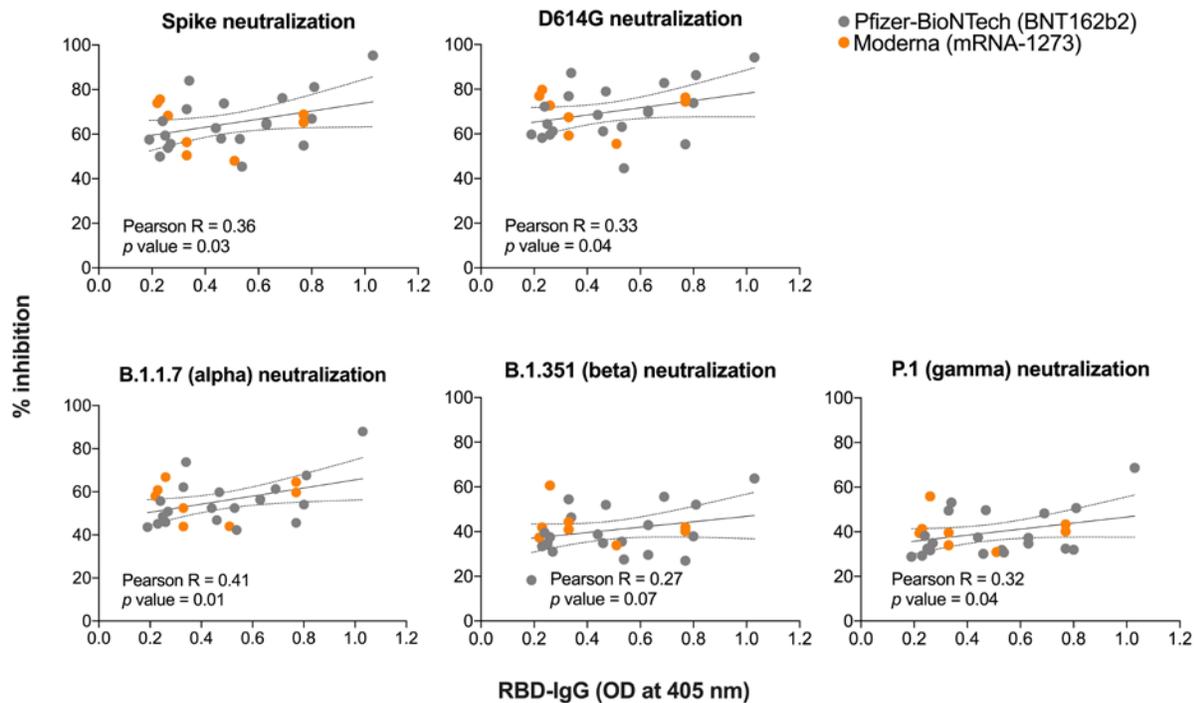
### Pre-pandemic samples



Narayanaswamy V, Pentecost B, Schoen CN, Alfandari D, Schneider SS, Baker R, et al. Neutralizing antibodies and cytokines in breast milk after coronavirus disease 2019 (COVID-19) mRNA vaccination. *Obstet Gynecol* 2022;139. The authors provided this information as a supplement to their article.

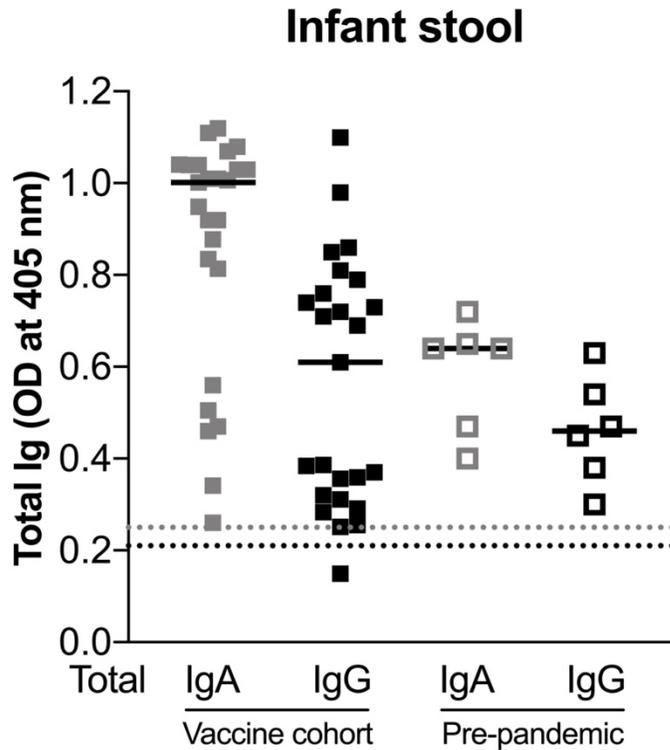
**Appendix 5. Weak correlation between anti-receptor-binding domain (RBD) immunoglobulin (Ig)G and percent inhibition of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike panel binding to ACE2. Neutralization ability was assessed in milk collected 21–23 days after the second dose using the MSD V-PLEX SARS-CoV-2 Panel 6 assay. The correlation between anti-RBD IgG and neutralization ability determined in the milk of 28 women. Variants of concern tested include D614G, Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1). 95% CIs, Pearson’s correlation coefficient (R) and P values are shown. ECL, electrochemiluminescence.**

% inhibition is calculated using the following equation  $1 - \frac{\text{Average Sample ECL signal}}{\text{Average ECL signal of the blank}} \times 100$



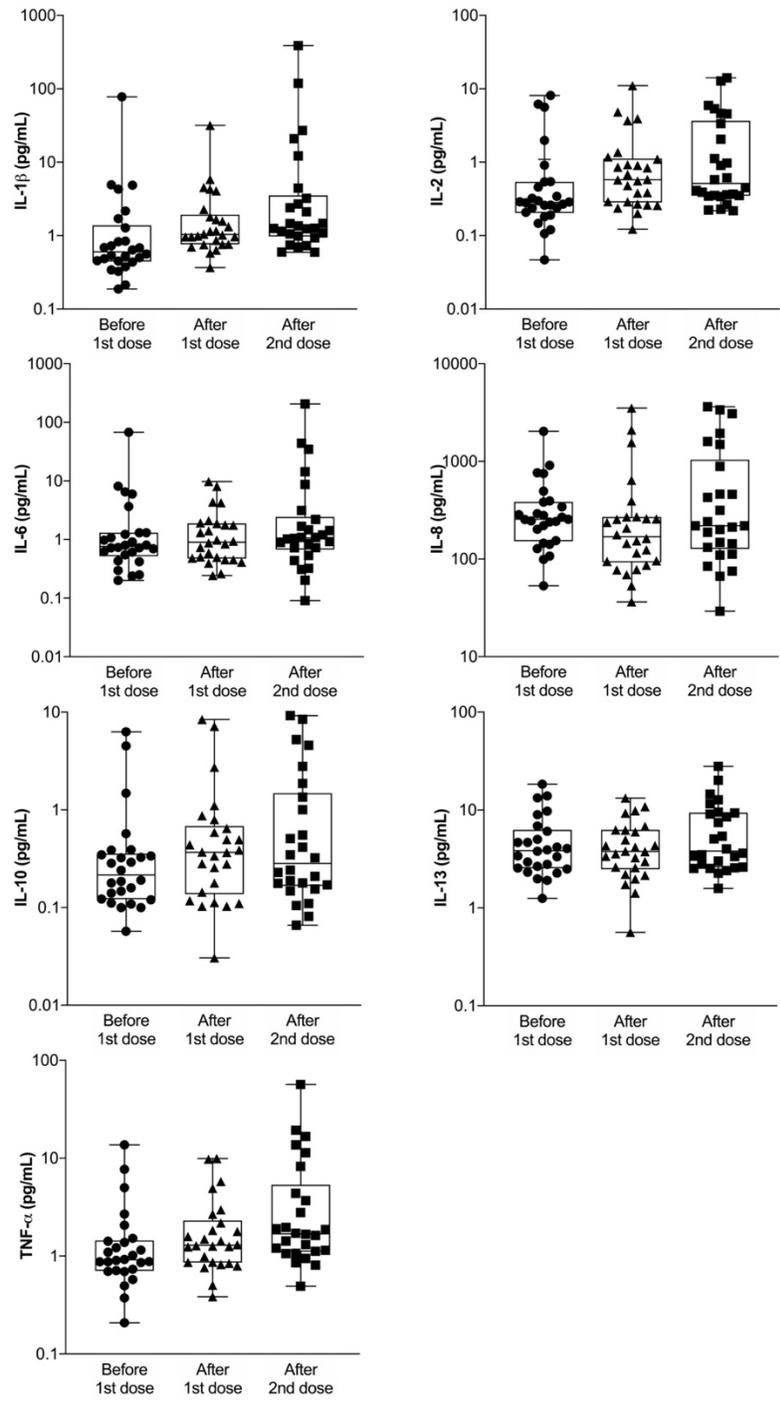
Narayanaswamy V, Pentecost B, Schoen CN, Alfandari D, Schneider SS, Baker R, et al. Neutralizing antibodies and cytokines in breast milk after coronavirus disease 2019 (COVID-19) mRNA vaccination. *Obstet Gynecol* 2022;139. The authors provided this information as a supplement to their article.

**Appendix 6. Total immunoglobulin (Ig)A and IgG are present in infant stool samples in the vaccine cohort and pre-pandemic controls. Levels of total IgA and IgG in stool from infants of coronavirus disease 2019 (COVID-19) immunized mothers (*filled square*; n=24) and levels of total IgA and IgG in pre-pandemic infant stool samples (*open square*; n=6) detected by the enzyme-linked immunosorbent assay (ELISA). Dotted lines indicate positive cutoff values, IgA in gray and IgG in black. *Solid lines* indicate median ODs.**



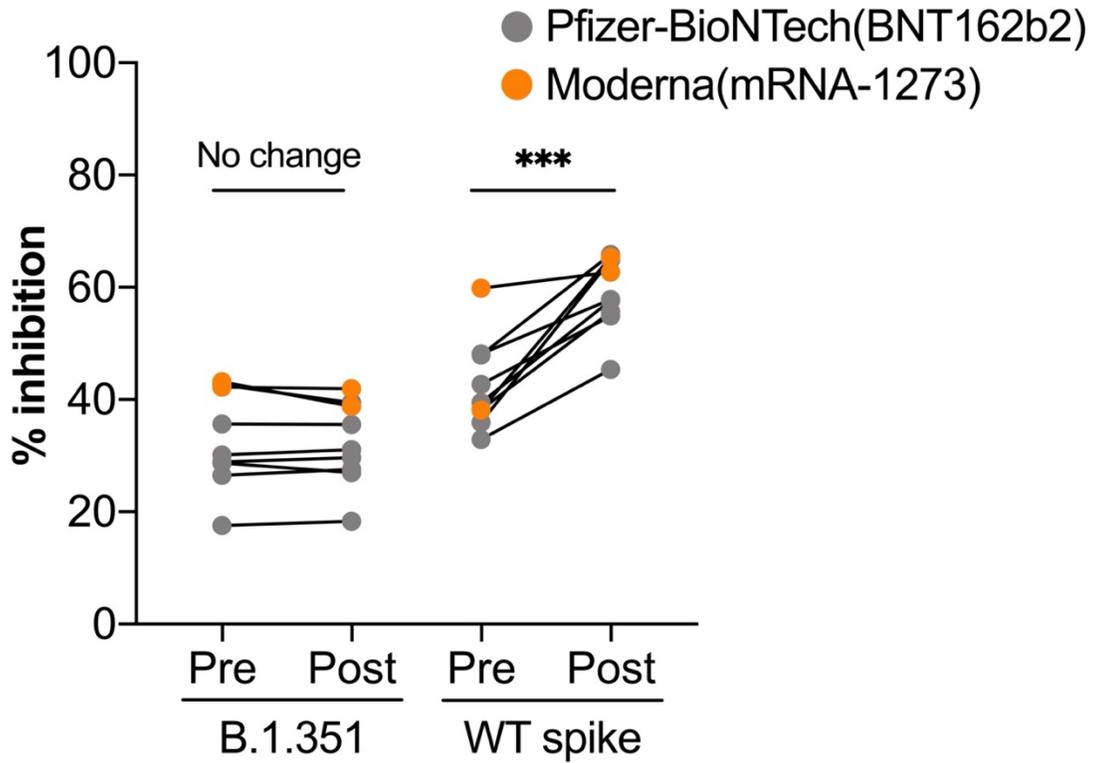
Narayanaswamy V, Pentecost B, Schoen CN, Alfandari D, Schneider SS, Baker R, et al. Neutralizing antibodies and cytokines in breast milk after coronavirus disease 2019 (COVID-19) mRNA vaccination. *Obstet Gynecol* 2022;139. The authors provided this information as a supplement to their article.

**Appendix 7. No significant change in levels of seven cytokines assayed in milk obtained before and after vaccination against coronavirus disease 2019 (COVID-19). Cytokine levels were assessed in milk samples obtained from 26 women prior COVID-19 immunization, after the first dose, and after the second dose. *Horizontal lines* in each bar indicate median concentration (pg/mL).**



Narayanaswamy V, Pentecost B, Schoen CN, Alfandari D, Schneider SS, Baker R, et al. Neutralizing antibodies and cytokines in breast milk after coronavirus disease 2019 (COVID-19) mRNA vaccination. *Obstet Gynecol* 2022;139. The authors provided this information as a supplement to their article.

**Appendix 8. Individual variability in the ability of postvaccine milk to neutralize spike and the Beta (B.1.351) variant. Milk from nine women who received an mRNA-based coronavirus disease 2019 (COVID-19) vaccine was able to neutralize the spike but not the Beta variant. A matched paired t-test was used to compare differences in percent inhibition between prevaccine and postvaccine milk samples. \*\*\* $P < .001$ .**



Narayanaswamy V, Pentecost B, Schoen CN, Alfandari D, Schneider SS, Baker R, et al. Neutralizing antibodies and cytokines in breast milk after coronavirus disease 2019 (COVID-19) mRNA vaccination. *Obstet Gynecol* 2022;139. The authors provided this information as a supplement to their article.