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COVID-19 vaccine response in pregnant and lactating women: a cohort study

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TITLE: COVID-19 vaccine response in pregnant and lactating women: a cohort study

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KJG has consulted for Illumina, BillionToOne, and Aetion outsite the submitted work. AF reported serving as a cofounder of and owning stock in Alba Therapeutics and serving on scientific advisory boards for NextCure and Viome outside the submitted work. GA reported serving as a founder of Systems Seromyx. MAE reported serving as medical advisor for Mirvie. All other authors report no conflicts of interest.

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CONDENSATION: COVID-19 vaccination confers a robust humoral response in pregnant and lactating women and immune transfer to neonates via placenta and breastmilk.

SHORT TITLE: COVID-19 vaccination in pregnancy and lactation

AJOG at a GLANCE:

A. Why was this study conducted? Because pregnant and lactating women were excluded from initial COVID-19 vaccine trials, data are lacking regarding vaccine efficacy and infant humoral protection in this population.

B. What are the key findings? Pregnant and lactating women elicited comparable vaccine-induced humoral immune responses to non-pregnant controls, and generated higher antibody titers than those observed following SARS-CoV-2 infection in pregnancy. Vaccine-generated antibodies were present in umbilical cord blood and breastmilk after maternal vaccination.

C. What does this study add to what is already known? This study provides the first data from a large cohort on maternal antibody generation in response to COVID-19 vaccination, compares vaccine-generated immunity to that from natural infection in pregnancy, and suggests vaccination of pregnant and lactating women can confer robust maternal and neonatal immunity.

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ABSTRACT

Background: Pregnant and lactating women were excluded from initial COVID-19 vaccine trials; thus, data to guide vaccine decision-making are lacking.

Objectives: To evaluate the immunogenicity and reactogenicity of COVID-19 mRNA vaccination in pregnant and lactating women compared to: (1) non-pregnant controls and (2) natural COVID-19 infection in pregnancy.

Study Design: 131 reproductive-age vaccine recipients (84 pregnant, 31 lactating, and 16 non-pregnant) were enrolled in a prospective cohort study at two academic medical centers. Titers of SARS-CoV-2 Spike and RBD IgG, IgA and IgM were quantified in participant sera (N=131) and breastmilk (N=31) at baseline, second vaccine dose, 2-6 weeks post second vaccine, and at delivery by Luminex. Umbilical cord sera (N=10) titers were assessed at delivery. Titers were compared to those of pregnant women 4-12 weeks from natural infection (N=37) by ELISA. A pseudovirus neutralization assay was used to quantify neutralizing antibody titers for the subset of women who delivered during the study period. Post-vaccination symptoms were assessed via questionnaire. Kruskal-Wallis tests and a mixed effects model, with correction for multiple comparisons, were used to assess differences between groups.

Results: Vaccine-induced antibody titers were equivalent in pregnant and lactating compared to non-pregnant women (median [IQR] 5.59 [4.68-5.89] pregnant, 5.74 [5.06-6.22] lactating, 5.62 [4.77-5.98] non-pregnant, $p = 0.24$). All titers were significantly higher than those induced by

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SARS-CoV-2 infection during pregnancy ($p < 0.0001$). Vaccine-generated antibodies were present in all umbilical cord blood and breastmilk samples. Neutralizing antibody titers were lower in umbilical cord compared to maternal sera, although this finding did not achieve statistical significance (median [IQR] 104.7 [61.2-188.2] maternal sera, 52.3 [11.7-69.6] cord sera, $p=0.05$). The second vaccine dose (boost dose) increased SARS-CoV-2-specific IgG, but not IgA, in maternal blood and breastmilk. No differences were noted in reactogenicity across the groups.

Conclusions: COVID-19 mRNA vaccines generated robust humoral immunity in pregnant and lactating women, with immunogenicity and reactogenicity similar to that observed in non-pregnant women. Vaccine-induced immune responses were significantly greater than the response to natural infection. Immune transfer to neonates occurred via placenta and breastmilk.

KEYWORDS: Antibodies; breastfeeding; breastmilk; cord blood; COVID-19 vaccine; maternal immunity, mRNA; neonatal immunity; pregnancy

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INTRODUCTION

More than 73,600 infections and 80 maternal deaths have occurred in pregnant women in the United States alone as of March 1, 2021[1]. SARS-CoV-2 infection is more severe in pregnant women compared to their non-pregnant counterparts, with an increased risk of hospital admission, ICU stay, and death[2]. Despite their higher risk, pregnant and lactating women were not included in any initial coronavirus disease 19 (COVID-19) vaccine trials, although the first vaccine trial began in pregnant women in February of 2021 (Pfizer/BioNTech, ClinicalTrials.gov Identifier: NCT04754594).

The COVID-19 pandemic has given rise to hundreds of vaccine platforms in development to fight SARS-CoV-2[3,4]. However, few of these platforms have been tested or are specifically designed to elicit immunity in vulnerable populations, including pregnant women. Pregnant women have long been left out of therapeutic and vaccine research, reportedly due to heightened safety concerns in this population[5–8]. Although the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM) encouraged the Food and Drug Administration (FDA) to include pregnant women in the COVID-19 vaccine emergency use authorization (EUA) due to the risk of increased disease severity in this population, evidence about vaccine immunogenicity to guide patient decision-making and provider counseling is lacking[9–11]. Specifically, given the novelty of the first emergency approved COVID-19 vaccines, both of which utilize mRNA to deliver SARS-CoV-2 Spike to educate the immune system[12,13], it remains unclear whether this novel vaccine approach will drive immunity in the context of pregnancy, and whether antibodies will be transferred efficiently to neonates via the cord and breastmilk. Here, vaccine-induced immunity was profiled

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in vaccinated pregnant, lactating and non-pregnant controls compared to women infected with SARS-CoV-2 during pregnancy.

MATERIALS AND METHODS

Study Design

Women at two tertiary care centers were approached for enrollment in an IRB-approved COVID-19 pregnancy and lactation biorepository study between December 17, 2020 and February 23, 2021. Eligible women were: (1) pregnant; (2) lactating; or (3) non-pregnant and of reproductive age (18-45); 18 years old, able to provide informed consent, and receiving the COVID-19 vaccine.

Participants and Procedures

Eligible study participants were identified by practitioners at the participating hospitals or were self-referred. A study questionnaire was administered to assess pregnancy and lactation status, history of prior SARS-CoV-2 infection, timing of COVID-19 vaccine doses, type of COVID-19 vaccine received (BNT162b2 Pfizer/BioNTech or mRNA-1273 Moderna/NIH), and side effects after each vaccine dose (injection site soreness, injection site skin reaction/rash, headache, myalgias, fatigue, fever/chills, allergic reaction, or other (reaction detailed). A cumulative symptom/reactogenicity score was generated by assigning one point to each side effect.

Sample Collection and Processing

Blood and breastmilk from lactating women were collected at: V0 (at the time of first vaccine dose/baseline), V1 (at the time of second vaccine dose/”prime” profile), V2 (2-6 weeks

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following the 2nd vaccine dose/"boost" profile), and at delivery (for pregnant participants who delivered during the study timeframe). Umbilical cord blood was also collected at delivery for pregnant participants. The V2 timepoint reflects full antibody complement, achieved one week after Pfizer/BioNTech and two weeks after Moderna/NIH[12,13]. Blood was collected by venipuncture (or from the umbilical vein following delivery for cord blood) into serum separator tubes. Blood was centrifuged at 1000g for 10 min at room temperature. Sera were aliquoted into cryogenic vials and stored at -80°C. Breastmilk was collected by the lactating participant into study-provided breastmilk bottles or breastmilk bags depending on volume. Breastmilk was centrifuged at 2000 rpm at 4°C for 25 minutes, supernatant was aliquoted into cryogenic vials and stored at -80°C.

Antibody Quantification

Antibody quantification was performed as described previously[14]. Briefly, a multiplexed Luminex assay was used to determine relative titer of antigen-specific isotypes and subclasses using the following antigens: SARS-CoV-2 Receptor Binding Domain (RBD), S1, and S2 (all Sino Biological), and SARS-CoV-2 Spike (LakePharma). Antigen-specific antibody titers were log10 transformed for time course analyses. PBS background intensity was reported for each antigen as a threshold for positivity. Titers resulting from natural infection and vaccination-induced antibodies against SARS-CoV-2 RBD and Spike were quantified from the same plate using ELISA as previously described[15]. Additional detail regarding antibody quantification may be found in Supplemental Methods.

Antibody Neutralization Assay

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On the morning of the experiment, 17,000 ACE2 cells were plated in each well of a flat-bottom 96-well plate in 100 µl of D10 (Dulbecco's Modified Eagle Medium (DMEM) +10% fetal bovine serum (FBS)). Six hours later, the serum samples were heat-inactivated by incubation at 56^{o}C for 1 hour. A solution containing virus at 1.9 ng equivalent of p24 per µl was prepared in D10. The heat-inactivated serum was diluted in this virus-containing media 1:5 fold and then 3-fold serial dilutions were done in the same virus-containing media. The virus and serum samples were incubated at 37^{o}C for 2 hours. 50 µl of the virus-serum mix was then added to the ACE2 cells. The lowest final dilution of each serum sample is therefore 15-fold. The cells were incubated at 37^{o} C for 48 hours, and the RFP was quantified using the flow cytometer (BD Accuri™ C6). Additional details about this assay may be found in the Supplemental Methods.

Statistical Analyses

Participant characteristics were summarized with frequency statistics. Continuous outcome measures were reported as either mean (standard deviation [SD]) or median (interquartile range [IQR]). Correlation analyses were performed using Spearman coefficients. Within and between group analyses of log10 transformed antibody levels in serum or breastmilk across multiple timepoints were evaluated by a repeated measures mixed effects (REML) model, followed by post-hoc Tukey's multiple comparisons test. Differences between paired maternal and cord sera IgG and neutralization titers were evaluated by Wilcoxon matched-pairs signed rank test. Statistical significance was defined as $p<0.05$. Statistical analyses were performed using GraphPad Prism 9 and Stata/IC version 16.1.

RESULTS

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From December 17, 2020 to March 2, 2021, samples were obtained from 131 enrolled participants: 84 pregnant, 31 lactating, and 16 non-pregnant reproductive-aged women. Of the pregnant vaccine recipients, 13 delivered during the study timeframe, and cord blood was collected at delivery from 10. Banked sera from 37 pregnant women infected with SARS-CoV-2 in pregnancy and enrolled between March 24, 2020 and December 11, 2020 were included as a second comparison group.

Participant characteristics

Participant demographic and clinical characteristics, sampling timepoints, and side effect profiles are presented in Table 1. The study population consisted primarily of White, non-Hispanic women, reflecting the healthcare worker population at the two hospitals. Five total participants reported prior SARS-CoV-2 infection: 2 pregnant, 2 lactating, 1 non-pregnant. Characteristics of the comparison group with natural SARS-CoV-2 infection in pregnancy are detailed in Supplemental Table 1. These participants all had symptomatic SARS-CoV-2 with known timing of infection.

Vaccination characteristics

At the time of the study, two COVID-19 vaccines had received EUA: Pfizer/BioNTech and Moderna. Both vaccines use mRNA to deliver the SARS-CoV-2 Spike antigen to the immune system[12,13], representing a novel vaccine platform never before tested in pregnancy. While mRNA vaccines have shown highly effective immune induction in non-pregnant adults, the immunogenicity and reactogenicity of this platform in pregnancy remains unclear. Equivalent numbers of pregnant women receiving the Pfizer/BioNTech and Moderna vaccines were included in our study. Of pregnant participants, the mean gestational age at first vaccine dose

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was 23.2 weeks, with 11 women (13%) receiving their first vaccine dose in the first trimester, 39 (46%) in the second trimester, and 34 (40%) in the third trimester. Side effect profiles between participant groups following vaccination were similar and are detailed in Table 1. The cumulative symptom score after the first dose in all three groups was low. After the second dose, there was no significant difference between groups with respect to cumulative symptom score (median (IQR) 2 (1-3), 3 (2-4), and 2.5 (1-4.5) in pregnant, lactating, and non-pregnant groups respectively, p = 0.40). Vaccine-related fevers/chills were reported by 32% (25/77) of pregnant women after the boost dose and 50% (8/16) of non-pregnant (p=0.25).

Delivery outcomes and characteristics of lactating women

Delivery information for the 13 pregnant participants who delivered during the study period is detailed in Table 2. All 13 were vaccinated in the third trimester. Three women delivered at hospitals other than the study sites and cord blood samples were not available. Of the ten umbilical cord blood samples available for analysis, 9/10 mothers had received both vaccine doses (median (IQR) 36.5 days (30-42) from first vaccine and 14 days (11-16) from second vaccine). One participant delivered 17 days after vaccine 1, with spontaneous preterm labor at 35 weeks' gestation. Lactating participant characteristics are detailed in Table 2.

The maternal vaccine response

IgM, IgG, and IgA responses to the Spike (S), receptor binding domain (RBD), S1-segment of S, and S2- segment of S were measured. A significant rise in all isotypes across all antigens was observed from V0 to V1, with a further rise in IgG levels from V1 to V2 in both the pregnant and lactating groups (**Fig 1A-D and Supplemental Fig 1**). Spike titers rose more rapidly than RBD-titers after the first (V1/prime timepoint) and second (V2/boost timepoint) vaccine dose, but the

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magnitude of the response did not differ across pregnant or lactating women. In contrast to IgG responses, IgM and IgA responses were induced robustly after the prime, and were poorly induced after boosting, across all groups (**Fig 1C and D**). Higher S- and RBD-specific IgA responses were noted in Moderna vaccinees compared to Pfizer/BioNTech vaccinees (**Supplemental Fig 2A-C**), potentially related to the extended boosting window used for the Moderna vaccine. By 2 weeks post-second vaccine, the dominant serum antibody response was IgG for pregnant, lactating, and non-pregnant women (**Fig 1E and Supplemental Fig 1C**). Vaccine-induced maternal antibody titers in sera did not differ by trimester of vaccination (**Supplemental Fig 3**). Strikingly higher levels of SARS-CoV-2 antibodies were observed in all vaccinated women compared to pregnant women with natural infection 4-12 weeks prior (**Fig 1F,** Kruskal Wallis $p<0.001$), highlighting the robust humoral immune responses induced by mRNA vaccination.

Impact of maternal vaccination on breastmilk antibody transfer

mRNA vaccination resulted in the induction of antibodies in the circulation of vaccinated women (**Fig 1**). However, whether these antibodies were transferred efficiently to infants remained unclear. Thus, we next examined the levels of antibodies in breastmilk of lactating mothers (**Fig 2 A-C**). Robust induction of IgG, IgA, and IgM were observed following the prime and boost. Interestingly, IgA and IgM levels did not increase with boosting, in synchrony with a minimal boost in these isotypes in serum (**Fig 1C/D and Supplemental Fig 1A-E**). However, a boost in breastmilk IgG levels was observed (**Fig 2A),** concomitant with the boost observed systemically/in maternal serum (**Fig 1A**). IgG1 RBD rose significantly from V0 to V2 (3.44 to 3.50, $p = 0.002$) but not V0 to V1 (3.44 to 3.45, $p=0.7$) in breastmilk, and there was no significant rise in anti-RBD IgA or IgM in breastmilk after either dose (**Supplemental Fig 4**).

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Overall these data suggest that the boost may drive enhanced breastmilk-transfer of IgG, in the setting of consistent unboosted IgA transfer.

Impact of maternal vaccination on placental antibody transfer

Maternal IgG is also capable of crossing the placenta to confer immunity to the neonate. Spike- and RBD-specific IgG were detectable in 10/10 umbilical cords after maternal vaccination (**Fig 2D/E**). The cord with the lowest Spike- and RBD-specific IgG belonged to a mother who delivered between the first and second vaccine doses and had received her first vaccine dose 17 days prior to delivery, suggesting that 2 doses may be essential to optimize humoral immune transfer to the neonate. Neutralizing antibody (NAb) titers were lower in umbilical cord than maternal serum, although this finding did not achieve statistical significance (**Fig 2F,** median [IQR] 104.7 [61.2-188.2] maternal sera, 52.3 [11.7-69.6] cord sera, p=0.05). Two umbilical cords had undetectable NAbs: in one case the mother had not yet received vaccine 2 (17 days from V1), in the other the mother was 7 days from boost dose. Interestingly, there was a significant improvement of transfer of S-, but not RBD-, specific IgG1 into the cord with time from boost (**Fig 2D/E**), suggesting that time from vaccination may be an important determinant of transfer rates of specific IgG subpopulations following immunization in pregnancy (**Supplemental Fig 5A/B)**.

Vaccine reactogenicity in pregnancy and lactation

Composite reactogenicity score after boost dose of vaccine was significantly positively correlated with both maternal serum and breastmilk antibody titers. Composite symptom score after vaccination was significantly positively correlated with maternal serum Spike- and RBD-specific IgG1 and IgG3, breastmilk anti-Spike IgG1, IgG3 and IgA, and breastmilk anti-RBD

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IgG1 (Supplemental Table 2). Within the pregnant women, medical comorbidities were not significantly associated with maternal serum antibody titers, although there were relatively few medical comorbidities in this group.

DISCUSSION

Principal Findings

Here, robust and comparable IgG titers were observed across pregnant, lactating, and non-pregnant controls, all of which were significantly higher than those observed in pregnant women with prior SARS-CoV-2 infection. Boosting resulted in augmented IgG levels in the blood, translating to transfer of IgG to the neonate through the placenta and breastmilk.

Results

The lack of boosting of IgM was likely related to an expected class switching to IgG, observed with increasing IgG titers observed following the boost. Conversely, the lack of boosting of IgA observed across all women in this study was unexpected. This lack of IgA augmentation may be related to the intramuscular administration of the vaccine, which triggers a robust induction of systemic, but not mucosal, antibodies. However, higher levels of IgA were noted after the boost in pregnant Moderna recipients, potentially attributable to enhanced class switching following a longer boosting interval. Robust IgG levels were noted in all vaccinees, and vaccine-induced IgG was transferred across the placenta to the fetus, as has been noted in the setting of influenza, pertussis, and other vaccination in pregnancy[16–18]. The presence of neutralizing antibody transfer in nearly all cords, and improved transfer with increased time from vaccination, points to the promise of mRNA vaccine-induced delivery of immunity to neonates. Transfer would perhaps be optimized if vaccination is administered earlier during gestation, though this needs to be directly

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examined in future studies. While the transferred levels of IgA through breastmilk did not increase with boosting, IgG transfer increased significantly with boost, resulting in the delivery of high levels of IgG to the neonate through breastmilk. Importantly, emerging data point to a critical role for breastmilk IgG in neonatal immunity against several other vaccinatable viral pathogens including HIV, RSV, and influenza[19–21]. In contrast, IgA dominates breastmilk profiles in natural SARS-CoV-2 infection[22]. The different isotype transfer profile for breastmilk (IgG in vaccine, IgA in natural infection) likely reflects differences in antibody profile programming across mucosally-acquired natural SARS-CoV-2 infection versus intra-muscular vaccination. Whether breastmilk IgG or IgA will be more critical for neonatal protection remains unclear.

Based on what is known about other vaccines, the amount of maternal IgG transferred across the placenta to the cord is likely to differ by trimester of vaccination[16,17]. Based on data from natural infection[14], qualitative changes in vaccine-elicited antibodies are likely to profoundly alter antibody transfer, and immunization with a de novo antigen earlier in pregnancy is likely to increase placental transfer. Understanding vaccine-induced antibody transfer kinetics across all pregnancy trimesters will be an important direction for future research. While timing maternal COVID-19 vaccination may not be possible during this phase of the pandemic, understanding optimal timing of vaccination to augment neonatal humoral immunity remains important. Unlike vaccines that aim to boost pre-existing antibodies (e.g influenza and pertussis vaccines), optimal timing for de novo vaccine administration remains unclear. Thus, as the prevalence of SARS-CoV-2 community spread decreases, different factors such as optimizing neonatal immunity via

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placental or breastmilk transfer may be weighted more heavily to inform future vaccine deployment.

Following EUA for the COVID-19 mRNA vaccines, safety information has been tracked by the CDC using the V-safe smartphone application. Consistent with our observations, the V-safe data indicate no significant differences in post-vaccination reactions in pregnant vs. non-pregnant women aged 16-54 years[23]. While the side effect profile of pregnant women receiving the COVID-19 vaccines was not significantly different from non-pregnant women, the relatively high incidence of fever (up to 32% following the second dose), raises a theoretical concern for pregnant recipients[24,25], although the level of risk remains controversial[26].

Clinical Implications

When considering vaccination in pregnancy, evidence regarding maternal and fetal benefit, as well as potential maternal and fetal harm and effects on pregnancy outcomes should be weighed carefully. While the absolute risk of severe COVID-19 is low in pregnant women, pregnancy is a risk factor for severe disease[27,28]. There are well-documented maternal, neonatal, and obstetric risks of SARS-CoV-2 infection during pregnancy[29–33]. These data provide a compelling argument that COVID-19 mRNA vaccines induce similar humoral immunity in pregnant and lactating women as in the non-pregnant population. These data do not elucidate potential risks to the fetus.

Research Implications

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Future studies, in larger populations spanning vaccine administration across all three trimesters and evaluating associated fetal/neonatal transfer of IgG via cord and breastmilk, may enhance our ability to develop evidence-based recommendations for the administration of vaccines, and particularly different platforms, during pregnancy. While limited evidence of antibody-dependent enhancement has been observed in the context of pre-existing natural or vaccine immunity in adults, future studies should carefully examine the impact of transferred immunity on infant immune response, and should define the optimal window for immunization to empower infants with robust immunity.

Strengths and Limitations

This study was limited by the select population of primarily healthcare workers from one US city, the focused time frame with limited number of delivered participants, inability to assess persistent immunity, and the exclusive focus on antibody titers rather than T cell-driven or other functional immunity. Future work examining T cells and other immune functions may provide additional insights on mRNA vaccine-induced immunity in pregnancy and lactation. The strengths of this work include: the provision of longitudinal data profiling vaccine-induced immune response across contemporaneously-recruited pregnant, lactating, and non-pregnant women; the ability to compare vaccine-induced IgG titers to those from prior SARS-CoV-2 infection; and the inclusion of 10 maternal/neonatal dyads, demonstrating transfer of vaccine-induced IgG (including NAbs) to the neonate, with improved cord titers achieved as interval from vaccination increased.

Conclusions

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COVID-19 vaccination in pregnancy and lactation generated robust humoral immunity similar to that observed in non-pregnant women with similar side effect profiles. While humoral immune response and side effects are only two of many considerations for pregnant women and their care providers in weighing whether or not to be vaccinated against COVID-19 in pregnancy, these data confirm that the COVID-19 mRNA vaccines result in comparable humoral immune responses in pregnant and lactating women to those observed in non-pregnant populations.

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GLOSSARY OF TERMS

SARS-CoV-2: a single-stranded RNA virus that causes COVID-19.

SARS-CoV-2 spike protein: a virus surface protein that mediates viral entry into cells and is composed of S1 and S2 subunits.

SARS-CoV-2 Receptor Binding Domain (RBD): a region of the spike protein that binds to the ACE2 (angiotensin-converting enzyme 2) receptor on human cells for viral entry into cells.

SARS-CoV-2 Nucleocapsid (N) antigen: an antigen important for eliciting antibodies against SARS-CoV-2 during infection. A critical protein in many parts of the viral life cycle.

COVID-19 mRNA vaccine: a vaccine designed by packaging messenger RNA (mRNA) that encodes for the SARS-CoV-2 spike protein into an injection. The mRNA elicits an immune response against the spike protein which allows a vaccinated individual's immune system to become trained to recognize the spike protein and prevent infection with SARS-CoV-2.

Antibody titers: a measurement of the antibody levels generated in response to exposure to an antigen.

Immunoglobulins (IgG, IgM, IgA): antibodies are referred to by immunoglobulin type, including IgG, IgM and IgA. IgG is the most abundant type of immunoglobulin-- it is found in all body fluids and can cross the placenta. IgM is primarily found in blood and lymph and is the first type of antibody to be generated in response to a new infection. IgA is found in mucous membranes including the respiratory and gastrointestinal tracts, as well as saliva and tears. IgA is the main type of antibody found in breastmilk.

Prime vaccine dose: the first dose of a vaccine that "primes" the body to respond to a subsequent exposure.

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Boost vaccine dose: an additional dose of vaccine given to "boost" the immune system. A boost dose is currently given for both approved COVID-19 mRNA vaccines 3-4 weeks after the prime vaccine dose.

Immunogenicity: the ability of a foreign substance (e.g., antigen or vaccine) to elicit an immune response in an individual.

Reactogenicity: the degree of physical effects following vaccination due to the body's immune response. These include the adverse reaction of fever and injection site soreness/ pain.

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524 **Table 1. Cohort Demographic Characteristics**

Characteristic	Non-pregnant (n=16), N (%)	Pregnant (n=84), N (%)	Lactating (n=31), N (%)
Participant age, mean (SD), y	38.4 (8.3)	34.1 (3.3)	34.6 (2.6)
Race			
White	12 (75%)	75 (89%)	27 (87%)
Black	2 (12%)	2 (2%)	0 (0%)
Asian	0 (0%)	6 (7%)	2 (6%)
Multi-racial	0 (0%)	1 (1%)	1 (3%)
Other	1 (6%)	0 (0%)	1 (3%)
Unknown	1 (6%)	0 (0%)	0 (0%)
Ethnicity			
Hispanic or Latino	0 (0%)	5 (6%)	2 (6%)
Not Hispanic or Latino	14 (88%)	79 (94%)	28 (90%)
Unknown/ not reported	2 (12%)	0 (0%)	1 (3%)
Maternal co-morbidities			
Chronic hypertension	1 (6%)	3 (4%)	3 (10%)
Diabetes/ gestational	0 (0%)	3 (4%)	3 (10%)
diabetes	2 (12%)	10 (12%)	3 (10%)
BMI > 30	2 (12%)	16 (19%)	7 (23%)
Asthma	0 (0%)	3 (4%)	0 (0%)
Immunosuppression / cancer			
Prior SARS-CoV-2 infection	1 (6%)	2 (2%)	2 (6%)
Vaccine type			
Pfizer-BioNTech	8 (50%)	41 (49%)	16 (52%)
Moderna	8 (50%)	43 (51%)	15 (48%)
Gestational age at 1st vaccine dose	n/a	23.2 (16.3, 32.1)	n/a
Trimester of 1st vaccine dose	n/a		n/a
- 1st		11 (13%)	
- 2nd		39 (46%)	
- 3rd		34 (40%)	
Timepoints for blood collection			
- Baseline/ at 1st dose (V0)	1 (6%)	31 (37%)	14 (45%)
- At 2nd dose (V1)	15 (94%)	78 (93%)	26 (84%)
- 2-5.5 weeks after 2nd dose (V2)	16 (100%)	17 (20%)	13 (42%)
Timepoints for milk collection			
- Baseline/ at 1st dose (V0)	--	3 (4%)	16 (52%)
- At 2nd dose (V1)	--	26 (31%)	28 (90%)
- 2-5.5 weeks after 2nd dose (V2)	--	0 (0%)	13 (42%)
Side effects at 1st vaccine dose[a]	12 (75%)	73 (88%)	20 (67%)

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- Injection site soreness	0 (0%)	1 (1%)	0 (0%)
- Injection site reaction/rash	5 (31%)	7 (8%)	9 (30%)
- Headache	2 (12%)	2 (2%)	4 (13%)
- Muscle aches	6 (38%)	12 (14%)	4 (13%)
- Fatigue	1 (6%)	1 (1%)	1 (3%)
- Fever/chills	0 (0%)	0 (0%)	0 (0%)
- Allergic reaction	2 (6%)	3 (4%)	0 (0%)
- Other[b]			
Side effects at 2nd vaccine dose[c]	12 (75%)	44 (57%)	17 (61%)
- Injection site soreness	0 (0%)	1 (1%)	0 (0%)
- Injection site reaction/rash	6 (38%)	25 (32%)	11 (39%)
- Headache	7 (44%)	37 (48%)	16 (57%)
- Muscle aches	9 (56%)	41 (53%)	14 (50%)
- Fatigue	8 (50%)	25 (32%)	12 (43%)
- Fever/chills	0 (0%)	1 (1%)	0 (0%)
- Allergic reaction	2 (12%)	7 (9%)	7 (25%)
- Other[d]			

[a]Not all participants provided side effect data after first dose: 2 patients (1 pregnant, 1 lactating) did not provide information. Percentages are thus based off of N=16 non pregnant, N=79 pregnant, and N=30 lactating participants

[b] "Other" side effects reported after vaccine dose 1: elevated heart rate, joint pain, nausea, swollen lymph node, sore throat

[c] Not all participants received the second dose at the time of analysis; N=16 non-pregnant, N=80 pregnant, and N=29 lactating patients received second dose. Of those who received second dose, 4 did not provide side effect data (N=3 pregnant, N=1 lactating). Percentages are thus based off of N=16 non pregnant, N=77 pregnant, and N=28 lactating participants.

[d] "Other" side effects reported after vaccine dose 2: joint pain, nausea, sore throat, dizziness/light headedness, stomach ache, night sweats, clogged ears, swollen eyes

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537 **Table 2. Characteristics of Pregnant, Delivered Vaccine Recipients and Lactating Vaccine Recipients**

Pregnant, Delivered Vaccine Recipients (N=13)	
Characteristic	**N (%)**
Gestational age at delivery, median, (IQR), wk	39.3 (39, 40.3)
Days from first vaccine to delivery, median (IQR)	36.5 (30, 42)
Days from second vaccine to delivery, median (IQR)[a]	14 (11, 16)
Labor	11 (85%)
Mode of delivery	
Vaginal	10 (77%)
Cesarean	3 (23%)
Birthweight, g	3452 (563)
Adverse pregnancy outcome	
Fetal growth restriction	0 (0%)
Preeclampsia/gestational hypertension	0 (0%)
Preterm delivery	1 (8%)
- Spontaneous	1
- Medically-indicated	0
Composite infant morbidity[b]	
Supplemental oxygen/ CPAP	1 (8%)
Transient tachypnea of the newborn (TTN)	1 (8%)
Special care nursery admission	0
NICU admission	2 (15%)
Respiratory distress syndrome	0
Necrotizing enterocolitis	0
Sepsis	0
Assisted ventilation	0
Seizure	0
Grade 3/4 intraventricular hemorrhage	0
Death	0
Lactating Vaccine Recipients (N=31)	
Characteristic	**N (%)**
Months after delivery, median (IQR)	7.3 (3.8, 10.8)
Months after delivery	
0-3	5 (16%)
3-6	6 (19%)
6-9+	18 (58%)
Unknown	2 (6%)

538 [a]2 patients delivered prior to receiving the second dose (17 days after V1 and 14 days after V1, cord
539 blood only available for the patient delivering 17 days after V1)
540 [b]The 1 preterm delivery accounted for the documented cases of supplemental oxygen, TTN, and 1 of the
541 2 NICU admissions. The other NICU admission was a term infant with growth restriction admitted for
542 persistent hypoglycemia.
543

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FIGURE LEGENDS

Figure 1. Maternal vaccination induces a robust SARS-CoV-2-specific antibody response

A-D. Violin plots show the log_{10} transformed mean fluorescence intensity (MFI) for **(A)** IgG Spike-, **(B)** IgG RBD-, **(C)** IgA Spike-, and **(D)** IgA RBD-specific titers across V0, V1, and V2 time points collected from non-pregnant reproductive-age (blue), pregnant (orange), or lactating (purple) participants. Participants who received BNT 162b2 from Pfizer/BioNTech are depicted as open circles, and participants who received mRNA-1273 from Moderna/NIH are depicted as closed circles. Differences across timepoints and groups were assessed by repeated measures mixed-effects model followed by posthoc Tukey's multiple comparisons test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

E. Line graph showing the log_{10} transformed relative Spike-specific titers across V0, V1, and V2 time points collected from non-pregnant (blue), pregnant (orange), or lactating (purple) participants for IgG (circles:solid lines), IgM (open triangles:dashed lines), and IgA (squares:dotted lines).

F. Violin plots show the IgG and IgM Spike-specific titer in non-pregnant (blue), pregnant (orange), lactating (purple), and naturally-infected pregnant (yellow) participants. Participants who received BNT 162b2 from Pfizer/BioNTech are depicted as open circles, and participants who received mRNA-1273 from Moderna/NIH are depicted as closed circles. Differences across groups were assessed by Kruskal-Wallis test followed by posthoc Dunn's multiple comparisons test. **** $p < 0.0001$ compared to natural infection in pregnant women.

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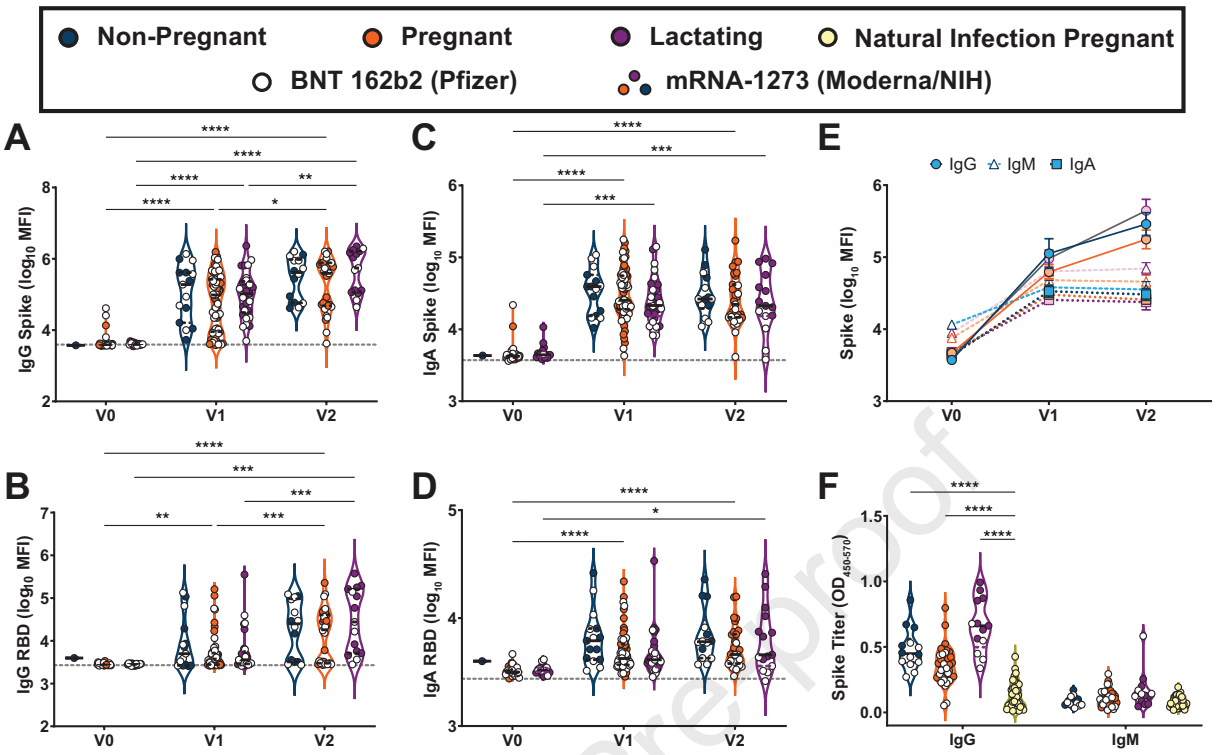
Figure 2. Maternal vaccination induces SARS-CoV-2-specific antibodies that transfer to breastmilk and umbilical cord blood

A-C. Violin plots show the log_{10} transformed mean fluorescence intensity (MFI) for **(A)** IgG1, **(B)** IgA, and **(C)** IgM Spike-specific breastmilk titers across V0, V1, and V2 time points. Differences across timepoints were assessed with repeated measures mixed effects model followed by posthoc Tukey's multiple comparisons test. Participants who received BNT 162b2 from Pfizer/BioNTech are depicted as open circles, and participants who received mRNA-1273 from Moderna/NIH are depicted as closed circles.

* p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001.

D-E. Dot plots showing relative **(D)** Spike- and (E) RBD-specific maternal blood (M) and cord blood (C) titers of IgG1. Wilcoxon matched-pairs signed rank test was performed to determine significance. TR: transfer ratio. On the right of each panel, the x axis shows the time from 2nd vaccine until delivery and the y axis shows cord blood log_{10} transformed titer for **(D)** IgG Spike (purple) and **(E)** IgG RBD (turquoise). Correlation was determined by Spearman correlation test. PBS Background subtraction was used to determine corrected optical density (OD) of 0.0.

F. Neutralizing antibody titers (50% inhibitory dose (ID50)) of maternal blood (M) and cord blood (C) are presented. Wilcoxon matched-pairs signed rank test was performed to determine significance.



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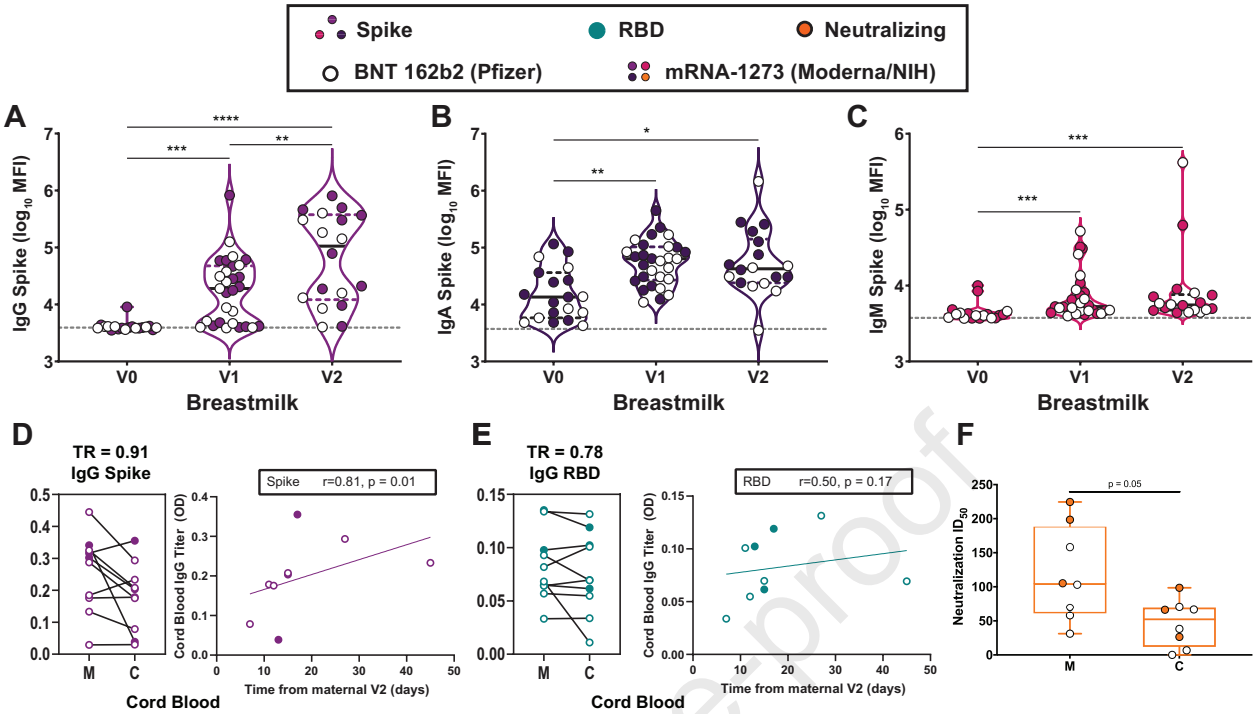

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