SARS-CoV-2 Antibodies in Breast Milk After Vaccination

Dolores Sabina Romero Ramírez, MD,a,,** María Magdalena Lara Pérez, MD,a,** Mercedes Carretero Pérez, PharmD, MSc, MPH,a María Isis Suárez Hernández, CNM,a Saúl Martín Pulido, MSN,a Lorena Pera Villacampa, CNM,a Ana María Fernández Villar, CNM,a Mónica Rivero Falero, MD,a Paloma González Carretero, MD,a Beatriz Reyes Millián, MD,a Sabine Roper, MD,a Miguel Ángel García Bello, Msc,a

**Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; and University of La Laguna, San Cristóbal de La Laguna, Spain

*Contributed equally as co-first authors

Dr Romero Ramírez conceptualized and designed the study, coordinated and supervised data collection, drafted the initial manuscript, and revised the manuscript; Dr Lara Pérez and Ms Carretero Pérez drafted the initial manuscript, coordinated and supervised data collection, and revised the manuscript; Ms Suárez Hernández and Ms Fernández Villar conceptualized and designed the study and revised the manuscript; Mr Pulido Hospital, Ms Pera Villacampa, and Drs Rivero Falero, González Carretero, and Reyes Millián designed the data collection instruments, collected data, and reviewed and revised the manuscript; Dr Roper translated and critically revised the manuscript; Mr García Bello worked in statistical analysis and interpretation of data and revised the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

WHAT’S KNOWN ON THIS SUBJECT: Recently, there have been 2 studies published on the presence of antibodies against severe acute respiratory syndrome coronavirus 2 in human milk after vaccination of the breastfeeding mother.

WHAT THIS STUDY ADDS: The interesting finding was the greater impact of vaccination on immunoglobulins in human milk with lactations > 23 months. The influence of the lactation period on immunoglobulins was specific and independent of other variables.


Background and Objectives: Passive and active immunity transfer through human milk (HM) constitutes a key element in the infant’s developing immunity. Certain infectious diseases and vaccines have been described to induce changes in the immune components of HM.

Methods: We conducted a prospective cohort single-institution study from February 2 to April 4, 2021. Women who reported to be breastfeeding at the time of their coronavirus disease 2019 (COVID-19) vaccination were invited to participate. Blood and milk samples were collected on day 14 after their second dose of the vaccine. Immunoglobulin G (IgG) antibodies against nucleocapsid protein as well as IgG, immunoglobulin M and immunoglobulin A (IgA) antibodies against the spike 1 protein receptor-binding domain against severe acute respiratory syndrome coronavirus 2 (anti-SARS-CoV-2 RBD-S1) were analyzed in both serum and HM samples.

Results: Most of the participants (ie, 94%) received the BNT162b2 messenger RNA COVID-19 vaccine. The mean serum concentration of anti-SARS-CoV-2 RBD-S-IgG antibodies in vaccinated individuals was 3379.6 ± 1639.5 binding antibody units per mL. All vaccinated study participants had anti-SARS-CoV-2 RBD-S1-IgG, and 89% of them had anti-SARS-CoV-2 RBD-S-IgA in their milk. The antibody concentrations in the milk of mothers who were breastfeeding 24 months were significantly higher than in mothers with breastfeeding periods <24 months (P < .001).

Conclusions: We found a clear association between COVID-19 vaccination and specific immunoglobulin concentrations in HM. This effect was more pronounced when lactation periods exceeded 23 months. The influence of the lactation period on immunoglobulins was specific and independent of other variables.
Transfer of passive and active immunity through human milk (HM) is a key element in the infant protection against infections. The mucosa is the point of entry for at least 90% of microorganisms, so the immunomodulatory capacity conferred by HM is important from the neonatal period onward. Breastfed infants are better protected against different infectious diseases, such as gastroenteritis, otitis media, urinary tract infection, neonatal sepsis, and necrotizing enterocolitis, as well as respiratory infections, with a reduced frequency, duration, and risk of hospitalization than formula-fed infants. Protection through HM may go beyond cessation of breastfeeding, although not all the mechanisms are well known.

HM includes many bioactive factors, such as secretory immunoglobulin A (sIgA), secretory immunoglobulin M (sIgM), immunoglobulin G (IgG), oligosaccharides, maternal glycoproteins, cytokines, nucleic acids, and leukocytes, which promote the infant’s developing immunocompetence. Immunoglobulins are the most studied immunoprotective components in HM. sIgA is the main isotype and is considered dominant in protecting the infant’s mucosal surfaces without stimulating a substantial inflammatory response (ie, by intracellular neutralization, immune exclusion, and virus excretion). Second most abundant is pentameric sIgM, which activates the complement cascade and causes agglutination of recognized pathogens and innate immunologic activities. IgG represents a lower proportion (2%) of immunoglobulins in HM, the implication of which is partly still unknown. It appears to be involved in immune surveillance in the intestinal lumen by binding to antigens, phagocytizing them, and transporting these antigen-IgG complexes to the lamina propria to activate B cells and thus affect the adaptive response of the infant. In vitro models with HIV, IgG is able to prevent infection at the intestinal level. The impact of the cellular and biochemical composition of HM on infectious diseases in mothers and infants has been studied and described elsewhere. Changes in HM composition have also been observed after administration of certain vaccines during pregnancy or lactation.

Since March 11, 2020, when the World Health Organization declared the global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the world has focused on studying this virus and preventing its spread. SARS-CoV-2 is a single-stranded RNA-encapsulated virus, the infection of which can lead from an asymptomatic process to a severe, multisystemic disease termed coronavirus disease 2019 (COVID-19). Children of all ages are susceptible to infection with this virus, and even those with mild or asymptomatic symptoms appear to be involved in disease transmission.

At the beginning of the pandemic, there were doubts about the safety of breastfeeding by mothers infected with SARS-CoV-2. Some authorities recommended against it. The current global recommendation is to encourage breastfeeding because no such route of transmission has been demonstrated and its benefits outweigh the risks.

Studies on breast milk from mothers with COVID-19 and on HM donors during the pandemic have revealed the presence of anti-SARS-CoV-2 antibodies and their neutralizing capacity. This confers hope of protection for breastfed infants.

The first SARS-CoV-2 vaccines were given emergency use authorization by the US Food and Drug Administration and appeared less than a year after virus sequencing. The initial exclusion of breastfeeding mothers and children in clinical trials reveals the need for studies to provide scientific information on these groups. We designed this study on the basis of the hypothesis that vaccination against SARS-CoV-2 leads to antibody excretion into breast milk and passive antibody transfer to breastfed infants.

METHODS

Study Design and Population

In this study, we applied a prospective cohort design with a convenience sample of health care professionals who were breastfeeding their children at the time of vaccination against SARS-CoV-2. The exposed vaccinated group consisted of individuals vaccinated with either the BNT162b2 messenger RNA (mRNA) COVID-19 vaccine or the mRNA-1273 COVID-19 vaccine. All mothers at the Hospital Universitario Nuestra Señora de Candelaria who reported breastfeeding and 8 breastfeeding mothers from other institutions were included. From February 2 to April 4, 2021, 102 vaccinated potential study participants were invited for enrollment the day they were administered their second dose of vaccine. Four of them were excluded from final analyses (Fig 1) for COVID-19 symptoms at the time of vaccination, 1 for past SARS-CoV-2 infection, and 2 for presenting serum parameters also suggestive of past infection. Twenty-four nonvaccinated breastfeeding mothers without previous SARS-
CoV-2 infection were recruited as a control group to determine the threshold for the presence of SARS-CoV-2–specific antibodies in HM. All participants gave their signed consent. Any type of breastfeeding at any infant age was accepted. Epidemiological variables and risk factors for severe COVID-19 disease in mothers and infants were collected (Table 1). Participants with HIV infection, diseases or treatment that cause immunosuppression, previous infections, or ongoing symptoms compatible with COVID-19 at the time of recruitment were excluded. Of the vaccinated study participants, 92 (94%) received the BNT162b2 mRNA COVID-19 vaccine and 6 (6%) received the mRNA-1273 COVID-19 vaccine, with a mean time range between doses of 25 ± 2 and 28 ± 1 days, respectively.

**Procedures**
Maternal blood and milk sampling was scheduled on day 14 after the second dose of the vaccine.

Vaccines against COVID-19 introduce information from the spike glycoprotein receptor-binding domain (RBD) of SARS-CoV-2 and generate a humoral immune response with immunoglobulin A (IgA), IgG, and immunoglobulin M (IgM) antibody production against its S1 subunit with its binding region for human cells, but they do not generate antibodies against the SARS-CoV-2 nucleocapsid protein, which solely appear in infected patients and those who have had the disease. Individuals with serum IgG against the SARS-CoV-2 nucleocapsid protein (anti-SARS-CoV-2 N IgG-serum) were excluded from the study for previous SARS-CoV-2 infection.

The SARS-CoV-2 IgG ARCHITECT Abbott (Abbott, Chicago, IL) assay was used for anti-SARS-CoV-2 N IgG detection. IgM antibodies against the spike protein of SARS-CoV-2 (anti-SARS-CoV-2 S1 IgM-serum) were assessed with the SARS-CoV-2 IgM ARCHITECT Abbott assay. By default, data for both assays were expressed as qualitative positive or negative results (ie, the sample to positive [S/C] ratio), given in detail in the Supplemental Information.
IgG antibodies against the receptor-binding spike domain S1 subunit (anti-SARS-CoV-2 RBD-S1 IgG- serum) were determined with the SARS-CoV-2 IgG II Quant Abbott assay, and results were expressed as international standard units (unit of 1000 binding antibody units [BAUs] per mL).28 According to the manufacturer, anti-SARS-CoV-2 RBD-S1 IgG concentrations of >560.90 BAUs per mL correspond to a 95% probability (95% confidence interval [CI]: 78%–99%) of neutralization capacity, calculated by a plaque reduction equivalent to an inhibition of 50% of infection in cultured cells.

Blood extraction by venipuncture and milk collection were performed simultaneously. HM expression was conducted by the mothers in the hospital setting, usually in the morning, at least 1 hour after the last feeding, using an electric pump (Spectra S1; Uzinmedicare Co., LTD, Hwaseong-si, Republic of Korea) and disposable extraction systems (Beldico, Marche-en-Famenne, Belgium) equipped with 1-μm filters and nonreturn valves. The target amount for extraction, 20 to 30 mL, was collected in food-standard polypropylene containers. Specific IgG (anti-SARS-CoV-2 RBD-S1 IgG-HM) and IgM antibodies (anti-SARS-CoV-2 S1 IgM-HM) in HM were determined with the same techniques used for blood serum samples. IgA (anti-SARS-CoV-2 S1 IgA-HM) was analyzed with the anti-SARS-CoV-2 enzyme-linked immunosorbent assay (Anti-SARS-CoV-2 ELISA) (IgA) (Euroimmun, Lübeck, Germany). Results are reported by calculating the ratio of the extinction of the control or patient sample over the extinction of the calibrator, S/P ratio. Details are available in the Supplemental Information. The cutoff values in HM were calculated from the control milk samples as follows: mean + 2 SD. Thus, the cutoff was 0.12 BAUs per mL for IgG and the S/P ratio for IgA was 0.37. Following the manufacturer’s instructions, an S/C ratio of 1 was used as the cutoff for IgM in serum and HM samples.

The study was approved by the institutional review board.

### RESULTS

The clinical and demographic characteristics of the 98 vaccinated and the 24 control participants (Fig 1) are given in Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Group (n = 24)</th>
<th>Vaccinated Group (n = 98)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>34 (30.7–36.0)</td>
<td>36 (33.2–38.7)</td>
<td>&lt;.001</td>
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<td>BMI, median (IQR)</td>
<td>23.1 (21.4–22.2)</td>
<td>23.9 (20.7–26.7)</td>
<td>.41</td>
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<tr>
<td>Mother’s risk factors for severe COVID-19, n (%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Liver disease</td>
<td>1 (4)</td>
<td>1 (1)</td>
<td>.36</td>
</tr>
<tr>
<td>Autoimmune disorders</td>
<td>0 (0)</td>
<td>8 (8)</td>
<td>.36</td>
</tr>
<tr>
<td>Immunosuppressive or immunodeficient state,b</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Genetic disease, chronic lung disease, severe obesity, diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth wt, median (IQR), g</td>
<td>3300 (2940–3400)</td>
<td>3225 (2994–3054)</td>
<td>.62</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>4 (17)</td>
<td>11 (11)</td>
<td>.49</td>
</tr>
<tr>
<td>Gestational age, mean ± SD, wk</td>
<td>39.9 ± 1.1</td>
<td>39.3 ± 1.8</td>
<td>.20</td>
</tr>
<tr>
<td>Birth wt, mean ± SD, g</td>
<td>3500 (2940–3400)</td>
<td>3225 (2994–3054)</td>
<td>.62</td>
</tr>
<tr>
<td>Exclusive breastfeeding</td>
<td>10 (42)</td>
<td>28 (28)</td>
<td>.29</td>
</tr>
<tr>
<td>Breastfeeding duration, mean ± SD, mo</td>
<td>6.5 (2.7–13.7)</td>
<td>11 (5.0–20.7)</td>
<td>.04</td>
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<tr>
<td>Child’s risk factors for severe COVID-19, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant cardiac disease (eg, heart failure, congenital heart disease, cardiomyopathies, and pulmonary hypertension)</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Cystic fibrosis, bronchopulmonary dysplasia, moderate to severe asthma, oxygen therapy, or CPAP therapy</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Immunosuppressive or immunodeficient state,b</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Cancer, chemotherapy, immunomodulators, radiotherapy, immunosuppressants, or corticosteroids (eg, &gt;20 mg/d of prednisone or equivalent) for &gt;14 d in the last 6 mo or immunoglobulins in the last 3 mo</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

CPAP, continuous positive airway pressure; IQR, interquartile range; —, not applicable.

a BMI at the time of screening calculated as wt (in kilograms) divided by height (in meters squared).
b Cancer, chemotherapy, immunomodulators, radiotherapy, immunosuppressants, or corticosteroids (eg, >20 mg/d of prednisone or equivalent) for >14 d in the last 6 mo or immunoglobulins in the last 3 mo.
Immunogenicity

We detected anti-SARS-CoV-2 N IgG-serum antibodies in 2 vaccinated participants, who were, therefore, excluded for previous SARS-CoV-2 infection. Serum samples were obtained from 97 enrolled individuals on day 14 ± 0.7 after the second dose of the vaccine. The mean SARS-CoV-2 RBD-S1 IgG-serum antibody concentration in vaccinated participants was 3379.64 ± 1639.46 IU/mL (95% CI: 3049–3710). Neutralizing antibody titers, as defined by the manufacturer, were >560.9 IU/mL in all vaccinated individuals. Two weeks post vaccination, 22.5% of the samples (95% CI: 14.3–32.5) were positive for anti-SARS-CoV-2 S1 IgM-serum. We did not find a significant correlation between antibody levels in serum and maternal age or maternal BMI. Serum samples of the control individuals were negative for anti-SARS-CoV-2 N and SARS-CoV-2 spike RBD IgM and IgG antibodies.

Antibodies in Breast Milk

The mean anti-SARS-CoV-2 RBD-S1 IgG level in the HM from the vaccinated participants was 12.19 ± 11.74 IU/mL (95% CI: 9.77–14.60; P = .001) and, therefore, lower than that in serum samples, but it was significantly higher than were the levels in the milk from the control group (0.02 ± 0.05 IU/mL [95% CI: 0.01–0.05; P < .001]). All vaccinated participants had anti-SARS-CoV-2 RBD-S1 IgG in their milk (95% CI: 96–100; Fig 2).

We found a positive correlation (r = 0.36; 95% CI: 0.17–0.53; P < .001) between anti-SARS-CoV-2 RBD-S1 IgG in serum and HM samples, which was stronger with breastfeeding periods <24 months (r = 0.67; 95% CI: 0.52–0.78; P < .001) than with breastfeeding periods ≥24 months (r = 0.32; 95% CI: 0.16–0.67; P = .19; Fig 3).

However, the difference in the serum to milk IgG correlations between the 2 time frames (<24 months and ≥24 months) was not statistically significant (P = .06). Consequently, we cannot conclude that the serum to HM correlation of SARS-CoV-2 RBD-S1 IgG differed between the 2 time ranges.

We also found anti-SARS-CoV-2 S1 IgA in 89% of the HM samples (95% CI: 81–95). A strong positive correlation was observed between anti-SARS-CoV-2 S1 IgA-HM and anti-SARS-CoV-2 RBD-S1 IgG-HM (r = 0.75; 95% CI: 0.65–0.83; P < .001; Fig 3). We did not detect anti-SARS-CoV-2 S1 IgM in HM (95% CI: 2–5).

Furthermore, we did not find any maternal age- or BMI-related differences in HM immunoglobulins.

Breastfeeding Period Related Effects of the COVID-19 Vaccination

Regarding the characteristics of the vaccinated study participants, we did not detect significant differences related to their breastfeeding periods (0–6, 6–12, 12–24, ≥24 months). We only observed differences related to the type of breastfeeding, for example, exclusive breastfeeding was more frequent in infants <6 months (Supplemental Table 2).

When analyzing immunoglobulin levels by the mentioned subgroups (Supplemental Table 3), we observed significant differences (P < .001) between breastfeeding periods of <24 months (group A) and ≥24 months (group B), with higher anti-SARS-CoV-2 immunoglobulin levels in group B. The anti-SARS-CoV-2 S1 IgA-HM S/P ratio in
group A was 1.35 ± 1.17 (95% CI: 1.1–1.6), and it was 3.20 ± 2.14 (95% CI: 2.17–4.23) in group B. In group A, the anti-SARS-CoV-2 RBD-S1 IgG-HM level was 9.16 ± 7.22 BAUs per mL (95% CI: 7.50–10.84), and in group B, it was 24 ± 17.57 BAUs per mL (95% CI: 15.52–32.47) (Fig 4).

Our group observed that both breastfeeding for $\geq 24$ months and high anti-SARS-CoV-2 RBD-S1 IgG levels in serum samples predict high IgG levels in breast milk. In a linear and multiple regression model, these associations proved to be independent, that is, the effect of the HM-IgG concentrations during breastfeeding for $\geq 24$ months was not associated with the mother’s IgG levels in serum samples. Compared with a breastfeeding period of $<24$ months, lactation for $\geq 24$ months led to an increase in the mean anti-SARS-CoV-2 RBD-S1 IgG level in HM by 17.04 BAUs per mL (95% CI: 12.07–22.15; $P < .001$).

**DISCUSSION**

In our study, we found that all vaccinated mothers developed specific anti-SARS-CoV-2 RBD-S1 IgG antibodies in serum and milk samples. These data point to a possible route of infant protection against the virus. The secretion of specific antibodies in naturally immunized mothers has been related to protection against enteric diseases, such as *Campylobacter*, *Vibrio cholerae*, *Salmonella typhimurium*, norovirus, etc,29–32 as well as a decrease in respiratory infections.33,34 Other authors have already described the presence of specific IgA and IgG antibodies in HM of mothers infected with SARS-CoV-2. It seems that the predominant response is reflected in an even higher IgA titer, which correlates with the neutralizing capacity demonstrated in HM.24

Moreover, certain vaccines have been shown to induce changes in the protection-related composition of HM. In the randomized clinical trial by Jarvis et al,17 mothers vaccinated against influenza in the third trimester of pregnancy had higher levels of antibodies against influenza A in their HM during the first 6 months post partum than nonvaccinated mothers. This type of reaction was also seen in studies with other vaccines, such as the tetanus and pertussis and the antimeningococcal vaccine.19,35,36 For that reason, our group decided to assess potentially similar effects of SARS-CoV-2 vaccination.

In our study, we found a direct link between COVID-19 vaccination and specific immunoglobulins in HM. All the analyzed HM samples contained specific IgG antibodies, and 89% of them contained specific IgA antibodies. These findings are in line with other recent studies on vaccinated mothers.37,38 In breast milk, the predominant response to vaccination was observed for IgG, which the mentioned authors attribute to parenteral
administration. Our group observed a higher percentage of mothers with anti-SARS-CoV-2 RBD-S1 IgG in their milk, although we cannot compare quantification outcomes because a semiquantitative technique was used for anti-SARS-CoV-2 S1 IgA evaluation. What we did find was a strong positive correlation between anti-SARS-CoV-2 RBD-S1 IgG concentrations and the IgA S/P ratio in breast milk. We believe that this finding is more likely to be related to the immune response to the vaccine antigens rather than the route of administration. Brady et al reported an IgA-IgG response and neutralizing capacity of milk from breastfeeding mothers who had been administered a live-attenuated (intranasal) versus inactivated (intramuscular) influenza vaccine, although the response was stronger on parenteral administration. The authors concluded that the entry through the mucosa is not enough to elicit a high IgA response to this vaccine.

In our study, all mothers developed IgG antibodies against SARS-CoV-2 RBD-S1 in serum samples, with concentrations that, according to the manufacturer, predict a potentially neutralizing capacity. Moreover, we found a highly significant correlation between the antibody levels in serum samples and those in HM. Hence, serum antibody concentrations seem to predict the appearance of antibodies in HM. Moreover, serum antibody levels strongly correlated with those in HM in lactations of <24 months, although this statement should be interpreted with caution because the correlation between lactation periods of <24 months and...
immunoglobulins, lactoferrin, and lysozyme have been described in lactation periods of \( \geq 24 \) months and during the involution of the breast near weaning. This phenomenon may be particularly beneficial when there is a need for augmented local protection against infections and may favor sick infants returning to the breast during this period.\(^{45}\)

A limitation of this study was that we do not have access to biosafety level 3 facilities and, therefore, have not been able to perform in vitro plaque reduction neutralization tests, the gold standard for determining SARS-CoV-2 antibody deactivating effectiveness. However, ELISA RBD-based assays have been described as a valid alternative to assess the neutralization capacity of said antibodies in HM.\(^{46,47}\)

Another possible limitation of this study is the difference in breastfeeding periods between control and vaccinated participants. Only 2 mothers in the control group had extended lactation periods of \( \geq 2\) years. We do not consider this difference to be clinically relevant or interfere with the results of our study. The women in the control group had not suffered the disease and therefore did not present anti-SARS-CoV-2 antibodies in neither serum nor milk samples, regardless of their breastfeeding period. In our sample, overall antibody levels were rather homogeneous in the control group, and the 2 mothers who extended breastfeeding to \( \geq 24 \) months did not display high antibody levels in their milk.

**CONCLUSIONS**

BNT162b2 and mRNA-1273 COVID-19 vaccines generate immunity in vaccinated mothers and are associated with vaccine-specific immunoglobulin concentrations in HM. This effect persists in breastfeeding periods of \( \geq 2 \) years. Immunity from breastfeeding and its possible impact on infant protection from SARS-CoV-2 infection is a hope for breastfeeding girls and boys, for whom the prospect of vaccination in this pandemic is still a long way off.

There are only few studies on HM composition in breastfeeding periods of \( \geq 2 \) years, and its immunologic benefit is often underestimated. The stronger effect of COVID-19 vaccination on HM immunoglobulins in lactation periods \( \geq 2 \) years suggests a need to increase support and health policies that encourage such long breastfeeding periods in times of a pandemic. More studies are needed on how long these antibodies last in HM and on their implication in protecting the breastfeeding population over time.

**ACKNOWLEDGMENTS**

We thank each and every one of the medical laboratory scientists and technicains who performed the laboratory procedure and the team of vaccinators for their help in locating nursing mothers at the time of vaccination against SARS-CoV-2. We also thank all the families who participated in this study.

**ABBREVIATIONS**

BAU: binding antibody unit
CI: confidence interval
COVID-19: coronavirus disease 2019
HM: human milk
IgA: immunoglobulin A
IgG: immunoglobulin G
IgM: immunoglobulin M
mRNA: messenger RNA
RBD: receptor-binding domain
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
slgA: secretory immunoglobulin A
slgM: secretory immunoglobulin M
S/P: sample to positive
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