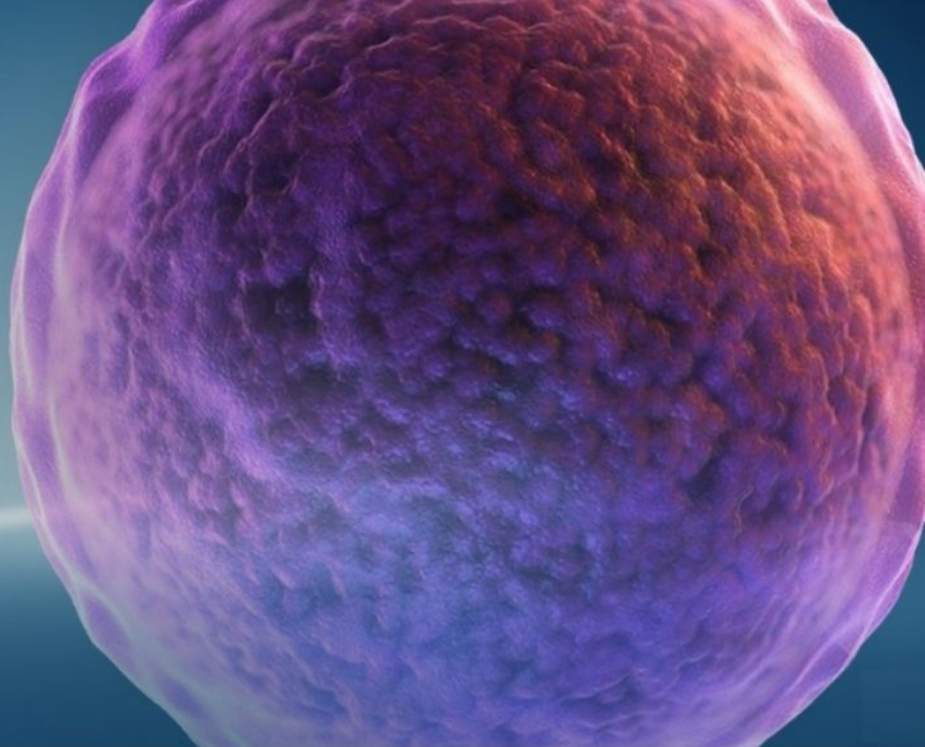


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# Detection of SARS-CoV-2 RNA and antibodies in breast milk of infected mothers

## 1 | INTRODUCTION

Human milk is the ideal food for babies because it provides immunological protection and important components for the growth and development of the baby.<sup>1</sup> However, some infections may be a contraindication for breastfeeding, for example, maternal HIV and primary CMV infection.<sup>2,3</sup>

Understanding the humoral response to SARS-CoV-2 infection in pregnant women and the degree to which this response is transferred across the placenta and breast milk, is essential for understanding COVID-19 protection in utero and after birth. The aim of our study is to evaluate the presence of viral RNA and antibodies to SARS-CoV-2 in breast milk.

## 2 | MATERIALS AND METHODS

The study analyzed 12 breast milk samples from mothers with positive swabs for SARS-CoV-2 admitted to the Department of Maternal Infantile and Urological Sciences of the Policlinico Umberto I in Rome between May and December 2021. Samples were collected at the time of delivery, regardless of the clinical picture and time of infection. At the same time, a maternal blood sample was taken.

The breast milk samples were subjected to the elimination of the lipid component by centrifugation and, subsequently, to an extraction protocol for the RNA. Specifically, the RNA was extracted using the Total RNA Purification Kit (NORGEN) from whole milk, skim milk, and lipid components. Subsequently, the viral load was assessed by real-time PCR (Altostar SARS-CoV2 RT-PCR Kit 1.5; ALTONA Diagnostics). For the detection of anti-nucleocapsid IgA antibodies was used an aliquot of whole milk and serum (Chorus SARS-CoV-2 IgA; Diesse), while for the detection of anti-Spike antigens IgM and IgG (DIASORIN LIAISON SARS-COV-2 TRIMERIC-S-IgG; LIAISON SARS-CoV-2 IgM) we used an aliquot of skimmed milk and serum.

## 3 | RESULTS

Twelve milk samples, in particular colostrum, from mothers with SARS-CoV-2 infection were analyzed. The average age of mothers was 27.2 years (interquartile range [IQR] 24.75–30). At the time of sample collection, the mean cycle threshold ( $C_T$ ) value of the nasopharyngeal swab was 27.9 (IQR 26.65–30.85). All milk samples

(whole, skim, and lipid components) showed negative results for SARS-CoV-2 RNA. Regarding antibodies in breast milk, eight (66%) samples were positive for anti-SARS-CoV-2 IgA with an average of 2.2 index (IQR 1.7–3.3) while no anti-SARS-CoV-2 IgM and IgG were found. Three of these eight women had only anti-SARS-CoV-2 IgG in serum and only one woman presented simultaneously serum antiviral IgM and IgG. The remaining four mothers with anti-SARS-CoV-2 IgA in their breast milk had no serum antibodies against it.

Finally, four mothers (34%) did not have any anti-SARS-CoV-2 antibodies in breast milk and serum, except one mother who had antiviral IgG and IgA in serum.

## 4 | DISCUSSION

The milk samples analyzed in our laboratory were negative for SARS-CoV-2 RNA. This data agrees with other studies in the literature<sup>4–6</sup> and supports the current indications of the WHO, which advises against breastfeeding only in severe cases of COVID-19 in the mother.<sup>7</sup> Breastfeeding is, on the contrary, encouraged because breast milk could be the vehicle of specific antibodies against the virus, inducing immune protection in the infant.<sup>8</sup> In particular, the IgA present predominantly in breast milk seems to induce a humoral response in the mucosa of the gastrointestinal tract, effective in preventing SARS-CoV-2 infection in the infant.<sup>9</sup> In our study, these immunoglobulins were found in 66% of the milk samples.

While, in disagreement with some studies in the literature,<sup>4,6,10</sup> anti-SARS-CoV-2 IgM and IgG were not found in any sample. However, in most of our patients (66%), anti-SARS-CoV-2 IgG and IgM were not detected in serum, suggesting that the samples were collected at an early stage of seroconversion, and this data could explain the lack of antibodies in milk.

This study has several limitations. First, a larger sample size is needed to characterize the SARS-CoV-2 humoral immunity in milk definitively. Second, there is the impossibility of evaluating the analytical results in relation to the days of maternal infection, due to the low prevalence of SARS-CoV-2 positive mothers and the difficulty in maintaining a follow-up over time.

Our findings suggest that SARS-CoV-2 appears not to be transmitted via breast milk so that infected mothers can breastfeed their newborns safely, in accordance with WHO guidelines. Indeed, the presence of anti-SARS-CoV-2 IgA antibodies in breast milk could protect the infant from infection as his immune system matures.

However, more evidence is needed to evaluate the precise role of breastfeeding in the transmission of SARS-CoV-2.

#### AUTHOR CONTRIBUTIONS

Marianna Calabretto conceptualized the study, analyzed samples, wrote, and drafted the manuscript. Rossella D'Alisa collected samples and clinical data and participated in drafting the manuscript. Sara Faraone and Laura Mazzuti participated in sample analysis. Francesco Pecorini supervised the collection of samples. Ombretta Turriziani supervised the study, reviewed, and approved the final manuscript before submission.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### ETHICS STATEMENT

Written consent was taken before enrollment and ethical approval was obtained from the Institutional Review Board (CE 6421 0163/2022).

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#### REFERENCES

1. Andreas NJ, Kampmann B, Le-Doare KM. Human breast milk: a review on its composition and bioactivity. *Early Hum Dev.* 2015;91(11):629-635.
2. Lawrence RM, Lawrence RA. Breast milk and infection. *Clin Perinatol.* 2004;31(3):501-528.
3. Lombardi G, Garofoli F, Manzoni P, Stronati M. Breast milk-acquired cytomegalovirus infection in very low birth weight infants. *J Matern Fetal Neonatal Med.* 2012;25(suppl 3):57-62.
4. Bäuerl C, Randazzo W, Sánchez G, et al. MilkCORONA study team. SARS-CoV-2 RNA and antibody detection in breast milk from a prospective multicentre study in Spain. *Arch Dis Child Fetal Neonatal Ed.* 2022;107(2):216-221.
5. Kumar J, Meena J, Yadav A, Kumar P. SARS-CoV-2 detection in human milk: a systematic review. *J Matern Fetal Neonatal Med.* 2021:1-8.
6. Pace RM, Williams JE, Järvinen KM, et al. Characterization of SARS-CoV-2 RNA, antibodies, and neutralizing capacity in milk produced by women with COVID-19. *mBio.* 2021;12(1):e03192-20.
7. World Health Organization. Breastfeeding and COVID-19. 2020. Accessed September 21, 2021. [https://www.who.int/publications/i/item/WHO-2019-nCoV-Sci\\_Brief-Breastfeeding-2020.1](https://www.who.int/publications/i/item/WHO-2019-nCoV-Sci_Brief-Breastfeeding-2020.1)
8. Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. *J Pediatr.* 2010;156(2 suppl):S8-S15.
9. Conti MG, Terreri S, Piano Mortari E, et al. Immune response of neonates born to mothers infected with SARS-CoV-2. *JAMA Netw Open.* 2021;4(11):e2132563.
10. Gao X, Wang S, Zeng W, et al. Clinical and immunologic features among COVID-19-affected mother-infant pairs: antibodies to SARS CoV-2 detected in breast milk. *New Microbes New Infect.* 2020;37:100752.