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The Levels of SARS-CoV-2 Specific Antibodies in Human Milk Following Vaccination

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines are being administered around the world; however, lactating women were excluded from SARS-CoV-2 vaccine trials. Therefore, knowledge about the effect of vaccination in this specific group is limited. This information is essential to empower lactating women to make a well-informed decision on their choice for vaccination. After natural infection, SARS-CoV-2 specific antibodies are present in human milk, which might offer protection for her newborn. The dynamics of these antibodies in human milk following vaccination remain to be elucidated.

Research Aim: To determine the effect of vaccination with BNT162b2 on the levels of SARS-CoV-2 specific IgA in human milk.

Methods: In this prospective longitudinal study, we included lactating women who received the BNT162b2 vaccine. Human milk samples were collected prior to vaccination and 3, 5, 7, 9, 11, 13, and 15 days after both vaccine doses. Samples were analyzed using enzyme-linked immunosorbent assay against the spike protein of SARS-CoV-2.

Results: In total, 366 human milk samples from 26 lactating women were analyzed. A biphasic response was observed, with SARS-CoV-2 specific immunoglobulin A (IgA) starting to increase between day 5 and 7 after the first dose of the vaccine. After the second dose, an accelerated IgA antibody response was observed.

Conclusion: After vaccination with the mRNA-based BNT162b2 vaccine, a SARS-CoV-2 specific antibody response was observed in human milk. The presence of SARS-CoV-2 specific IgA after vaccination is important as antibodies are transferred via human milk, and thereby might provide protection to infants against COVID-19.

Keywords

BNT162b2, breastfeeding, COVID-19, coronavirus, immunization, immunoglobulins, lactation, Pfizer, secretory IgA

Background

The year 2020 was dominated by the consequences of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. This novel coronavirus was declared a global pandemic on March 11, 2020, and the virus has now caused over 100 million cases of coronavirus disease 2019 (COVID-19) around the world, with 2 million deaths in less than 14 months (European Centre for Disease Prevention and Control, n.d.). In this past year, many preventive methods, such as social distancing and lockdowns, have been implemented worldwide to limit the spread of SARS-CoV-2 and protect vulnerable populations. Within a year, more than 200 vaccine candidates against SARS-CoV-2 were developed, with more than 50 vaccines tested in clinical trials (Belete, 2021). Pregnant and lactating women were not

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included in vaccination trials, even though those women and their newborns may be at risk of developing more severe COVID-19 (Adeyinka et al., 2021; Kim et al., 2020). Little is known about the effects of SARS-CoV-2 vaccines on pregnancy and lactation. On January 6, 2021, the Netherlands started vaccinating, including lactating women. Evidence-based guidelines for breastfeeding practices after vaccination are currently lacking.

The benefits of human milk have been long known. It has been demonstrated that human milk reduces morbidity and mortality compared to formula feeding, mainly due to a decrease in infections, including respiratory infections (Lamberti et al., 2013). In the first six months of life, the immune system of an infant is still developing, leaving them with a limited ability to produce an effective antibody response. Luckily, these antibodies are transferred from the mother to the child through human milk. The most abundant antibody found in human milk is secretory immunoglobulin A (IgA), which plays a major role in mucosal immunity as a first line of defense against many pathogens (Schlaudecker et al., 2013). IgA levels vary between women and are influenced by multiple factors, including lactation stage, gestational age at delivery, and maternal factors, including stress, food intake, and exercise (Moirasgenti et al., 2019; Saso & Kampmann, 2020; Urwin et al., 2012).

Specific SARS-CoV-2 antibodies with neutralizing capacity have been detected in human milk of COVID-19 recovered mothers (Fox et al., 2020; *van Keulen et al., 2021). A specific SARS-CoV-2 antibody response in human milk after vaccination has been demonstrated (Gray et al., 2021). However, since lactating women were excluded from vaccine trials because of safety reasons, knowledge about the levels of human milk antibodies after vaccination remains limited (Bardají et al., 2021).

BNT162b2 (Comirnaty®), developed by BioNTech/Pfizer, is an mRNA-based vaccine that encodes for the spike (S) protein of SARS-CoV-2. Antibody responses in serum after the first and second dose of BNT162b2 were reported in a phase 1/2 trial (Sahin et al., 2020). BNT162b is injected intramuscularly, focusing on systemic immune responses (IgG) and less so on mucosal immune responses (IgA; Poland et al., 2020). The IgA antibody response is not known for the mRNA-based vaccination BNT162b2. Especially for lactating women, it would be valuable to gain more insight into the IgA antibody response in human milk following vaccination since this could provide protection against COVID-19 for their infants.

Given the importance of breastfeeding on the health of infants, and for lactating women to make an informed decision about whether or not to be vaccinated, it is important to gain insights into the effect of SARS-CoV-2 vaccination on SARS-CoV-2 antibodies in human milk. This study aimed to determine the levels of specific IgA antibodies in human milk following the first and second dose of BNT162b2.

Key Messages

- Lactating women were excluded from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine trials. Therefore, knowledge about the influence of vaccination in this specific group is limited.
- After the SARS-CoV-2 vaccine (BNT162b2), SARS-CoV-2 specific antibodies are produced in human milk, following a biphasic pattern.
- The presence of SARS-CoV-2 specific IgA in human milk after vaccination is important as antibodies are transferred via human milk and might provide protection to infants against coronavirus disease 2019.
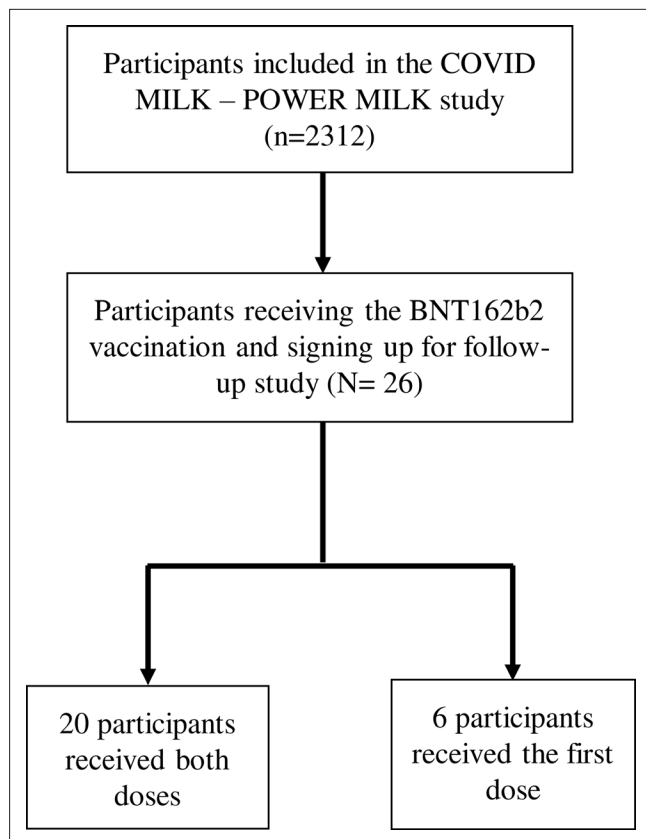
Methods

Research Design

The current study was an extension of the COVID MILK – POWER MILK study and was a prospective longitudinal study. This design enabled us to determine the levels of SARS-CoV-2 specific IgA in human milk following administration of the mRNA-based COVID-19 vaccine BNT162b2 (Comirnaty®, BioNTech/Pfizer, Mainz, Germany). Ethical approval was acquired from the Independent Ethics Committee (IEC) on January 18, 2021 (METc 2020.425). The study was conducted in accordance with the principles of the declaration of Helsinki and the ICH GCP Guidelines and the Regulation on Medical Research Involving Human Subjects.

Setting and Relevant Context

This study was conducted in the Netherlands. In 2018, around 70% of lactating women living in the Netherlands breastfed their child after birth and, of these women, 20% were still breastfeeding after six months (Engelse & Dommelen, 2020). Breastfeeding is promoted and supported by the Dutch Nutrition Centre Foundation, which is funded by the Dutch government (Theurich et al., 2019). The Netherlands' SARS-CoV-2 vaccination program started on January 6, 2021. At the beginning of the vaccination program, the BNT162b2 vaccination was given to a limited number of health care professionals and vulnerable populations, like the elderly (National Institute for Public Health and the Environment, 2021).

Sample

Participants from the COVID MILK – POWER MILK study, who were still breastfeeding their infant and were to receive a SARS-CoV-2 vaccination, could sign up for this follow-up study. All lactating women in the Netherlands could participate in the COVID MILK – POWER MILK study and were recruited via (social) media. There were no exclusion criteria. This study

Participants included in the COVID MILK – POWER MILK study (n=2312)

↓

Participants receiving the BNT162b2 vaccination and signing up for follow-up study (N= 26)

↓

20 participants received both doses

6 participants received the first dose

Figure 1. Flowchart of the Vaccination Study Embedded in the Larger COVID MILK – POWER MILK Study.

aimed to quantify the prevalence of milk conversion in the Netherlands during the winter of 2020–2021. Over 2300 women participated in the COVID MILK - POWER MILK study. The present vaccination follow-up study was embedded within this large study. We aimed to include 20 women who would donate pre-vaccination milk (see instructions below) and blood samples and who would receive two doses of the vaccine. In total, 26 women participated in the current study, of whom 20 received both doses of the vaccine, and six received the first dose (Figure 1). Due to the small sampling interval, 366 milk samples were collected, enabling us to provide a detailed overview of the levels of SARS-CoV-2 specific antibodies following vaccination.

Measurement

Characteristics of the participants were obtained by an online questionnaire. Before analysis, the collected human milk and serum samples were stored at the Amsterdam University Medical Center, location VUmc, at -80°C. To assess the SARS-CoV-2 specific IgA antibodies in human milk and IgG in serum, an enzyme-linked immunosorbent assay (ELISA) with the SARS-CoV-2 spike protein was used, as described previously (van Keulen et al., 2021). Soluble perfusion-stabilized spike protein of SARS-CoV-2 was generated and immobilized overnight on a 96-well plate (Greinier) using 0.1M NaHCO3

followed by a 1-h blocking step with 1% casein Phosphate Buffered Saline (PBS; Thermo Scientific). The human milk samples were diluted at 1:10, and the serum samples 1:50 in 1% casein PBS (Thermo Scientific) and incubated on the spike protein coated 96-well plates, for 2 hr to allow binding to the target protein. Finally, a 1:3000 diluted horseradish peroxidase (HRP)-labeled goat anti-human IgG (Jackson, Immunoresearch) in 1% casein PBS was used to detect specific IgG in the serum samples. In contrast, a 1:5000 diluted HRP-labeled goat anti-human IgA (Biolegend) in 1% casein PBS was used for the human milk samples. After 1 hr incubation, 3,3′,5,5′-Tetramethylbenzidin was used for the read-out at 450 nm. For the determination of the cut-off value, a relative operating characteristic curve analysis was performed for both milk and serum samples using pre-pandemic negative samples and polymerase chain reaction proven positive samples. The milk samples were considered positive at a OD450nm cut-off value of 0.502 and 0.452 for the serum samples. With these cut-off values, the sensitivity was 67.9% (95% CI [61.0, 74.1]) for IgA in human milk with a specificity of 99.0% (95% CI [94.7, 100.0]) and for serum IgG the sensitivity was 95.9 (95% CI [92.9, 97.6]) with a specificity of 99.1 (95% CI [94.9, 100]).
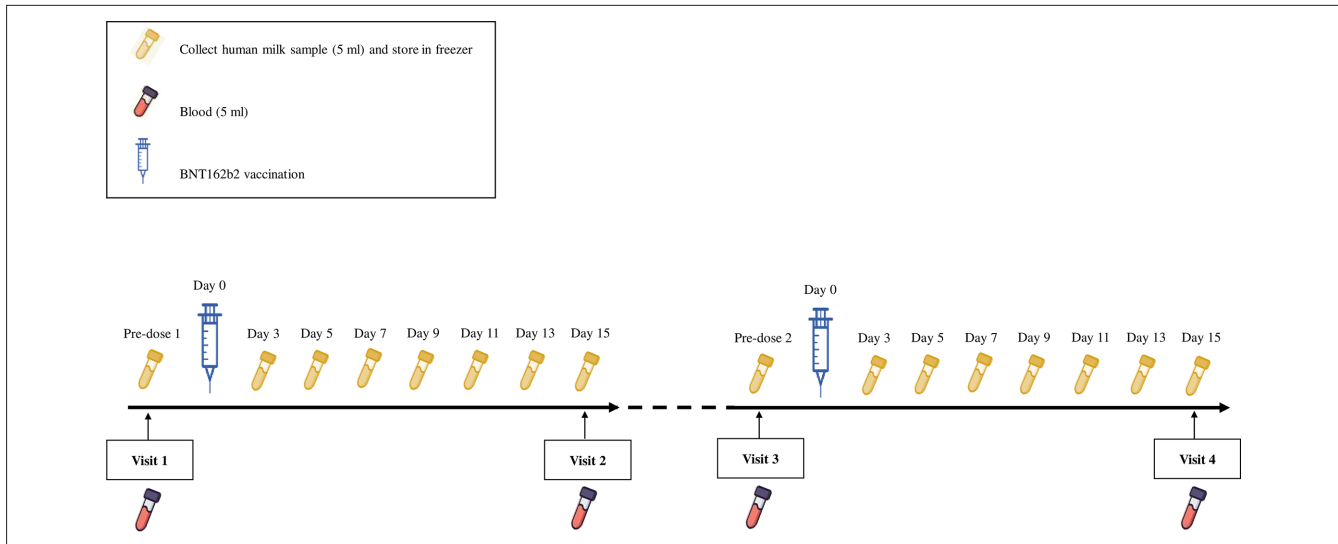
Data Collection

For the vaccination follow-up study, milk samples were collected longitudinally (Figure 2), between January 3, 2021 and March 9, 2021. Written informed consent was obtained from all participants. All scientific information collected as part of this study was treated confidentially and anonymized by assigning a unique code to each participant. All participants were requested to send their vaccination certificate including type of vaccination and lot number.

For each participant, 16 samples of human milk were collected according to a schedule: one sample before the first vaccination and one sample on days 3, 5, 7, 9, 11, 13, and 15–17 days after the first vaccination. This schedule was the same for the second vaccination. Participants were instructed to empty one breast in the morning, before the first feeding moment, and to collect 5 ml of milk after mixing the milk, then store the milk samples in their freezers.

Four blood samples were collected to determine the levels of systematically circulating IgG. The first sample was drawn before vaccination (for the original study), the second sample was collected 15–17 days after the first vaccination (second visit), the third sample was collected 1 day prior to the second vaccination (third visit), and the fourth sample was collected 15–17 days after the second vaccination.

Data Analysis

Data are reported in the descriptive statistics frequencies, mean with standard deviation (*SD*) or median with interquartile ranges (IQR), depending on the distribution. GraphPad Prism 9 for Windows was used to display the levels of IgA

Figure 2. Timeline of Data Collection.

antibodies in human milk after vaccination. To determine variability in individual immune response after vaccination, a quartile coefficient of dispersion (QCD) was calculated ([75th quartile – 25th quartile]/median). Statistical analyses were performed by using IBM SPSS Statistics for Windows, Version 27.

Results

Participants' characteristics are shown in Table 1. The participants had a median age of 34 years (IQR 31–35) and were breastfeeding for 7 months (IQR 5–9). Their mean body mass index was 23.9 (*SD* 3.5). In total, 366 human milk samples and 80 serum samples were collected. The 20 participants who received both vaccinations had a median follow-up

Table 1. Characteristics of Lactating Participants Receiving BNT162b2 Vaccine (*N* = 26).
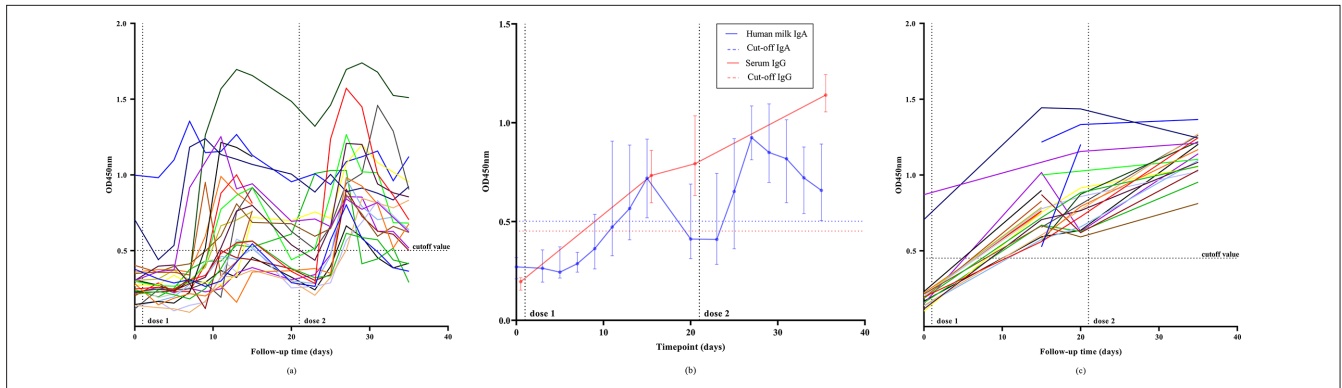
Characteristics	*n* (%)
Chronic disease[a]	3 (11.5%)
Parity (primiparous)	12 (46.2%)
Positive RT-PCR for COVID-19 prior to vaccination	1 (3.8%)
Vaccination history	
Child-immunization[b]	26 (100%)
Whooping cough vaccine during pregnancy	25 (96.2%)
Other[c]	14 (53.8%)

Note. RT-PCR = reverse transcription polymerase chain reaction.
[a]Diabetes Mellitus Type 1, epilepsy, hypothyroidism.
[b]According to the Dutch National Immunization Programme (National Institute for Public Health and the Environment, 2018).
[c]Other vaccinations including: Hepatitis B and A, yellow fever, rabies, typhoid fever, influenza A, and cholera.

time of 38 days (range, 35–45 days). Two of the participants had detectable SARS-CoV-2 specific IgA levels prior to vaccination. One of the participants had detectable SARS-CoV-2 specific IgG levels in her serum but did not show SARS-CoV-2 specific IgA antibodies in her milk before vaccination.

Antibodies in Human Milk

The individual SARS-CoV-2 specific antibody responses in human milk are displayed in Figure 3a. After vaccination, high inter-individual variability in SARS-CoV-2 specific IgA response in human milk was observed, with a QCD ranging from 0.29–1.23. The median SARS-CoV-2 specific antibody response in human milk is displayed in Figure 3b, showing a biphasic response in human milk. Human milk SARS-CoV-2 specific IgA started rising approximately 5–7 days after the first vaccination, showing an increase of 12% per day. On Day 15, SARS-CoV-2 specific IgA had increased approximately three-fold compared to baseline level, after which a turning point seemed to have been reached. Subsequently, between 15 days after the first dose and just before the second dose (Day 19–36) SARS-CoV-2 specific SARS-CoV-2 specific IgA levels decreased by 43% and stabilized at approximately 50% of the peak level. This level was sustained until approximately 3 days after the second dose. The second dose elicited a faster response than the first dose. The level of SARS-CoV-2 specific IgA increased by 2.3 times within a week compared to the level prior to the second dose. The peak level after the second dose was 1.3 times higher compared to the peak level after the first dose. Seven days after the second dose, SARS-CoV-2 specific IgA levels gradually declined, decreasing by 33% until the end of sample collection. In total, 35 days after the first dose of the vaccine,

Figure 3. Individual IgA response in human milk starting before vaccination post second vaccination. (b) Dynamics of median antibodies levels in human IgG response in serum starting before vaccination and continuing until 15 days. *Note.* Every colored line represents the dynamics in antibody level of an individual. Colors are the same for both figures. Error bars indicate the 95% confidence levels at each given time point. OD450nm = optical density using a microplate reader with a immunoglobulin A; IgG = immunoglobulin G.

SARS-CoV-2 specific IgA in human milk had increased by 2.4 times. This level was significantly higher compared to the baseline level ($p < .001$). Figure 4 shows the proportions of participants with milk and seroconversion. As demonstrated in this graph, most of the participants show milk conversion after the first dose of the vaccine, while some participants only show milk conversion after the second dose.

Antibodies in Serum

For each participant, four serum samples were obtained over the study period and a distinct pattern in SARS-CoV-2 specific antibody levels was observed compared to human milk. Serum SARS-CoV-2 specific IgG only showed a gradual increase over time and no declines (Figure 3b [median levels] and 3c [individual data]). In contrast to the IgA levels observed for milk, SARS-CoV-2 specific serum IgG showed

only limited variability, with a QCD ranging from 0.17 to 0.51. This inter-individual variability in serum is almost 2 times smaller than human milk ($p < .001$).

The steepest rise in serum SARS-CoV-2 specific IgG was seen in the first 2 weeks after the first dose. On Day 15, the level of SARS-CoV-2 specific IgG in serum had increased by 3.7 times compared to the level prior to the first dose. In the third week, the increase in serum SARS-CoV-2 specific IgG seemed to stabilize and, subsequently, a 1.4-fold increase was observed after the second dose. In total, after vaccination, SARS-CoV-2 specific IgG in serum had increased by 5.8 times over the study period. As shown in Figure 4, all participants showed seroconversion after the first dose of the vaccine.

Discussion

To empower lactating women to make an informed decision about whether to get vaccinated against COVID-19, more research is needed in this area. Literature about the immune response after vaccination against COVID-19 is limited in lactating women. Gray et al. (2021) examined the antibody response by collecting three human milk samples of lactating women ($N = 31$) and showed only an increase in SARS-CoV-2 specific human milk antibodies after the first dose and not after the second dose. This is not in accordance with our results, we observed an increase after both doses, which could be explained by differences in the timing of sample collection. Gray and colleagues only collected human milk samples once after each dose, making it impossible to determine the dynamics of antibodies in human milk in detail. Therefore, they might have missed the increase and subsequent decrease in antibody levels, as we observed. Other preliminary studies were also limited by the timing of sample collection and sample sizes (Friedman et al., 2021*; Golan et al., 2021).
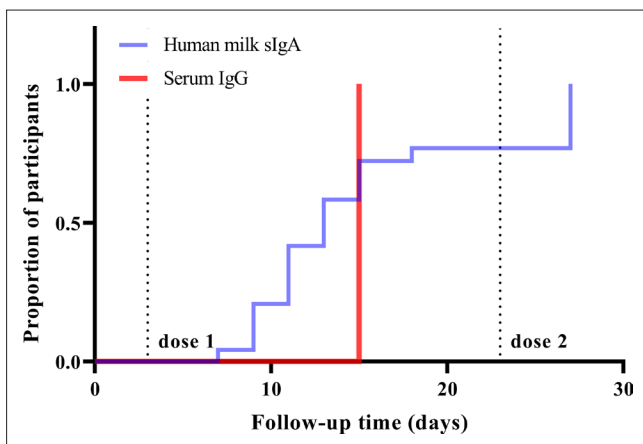
Figure 4. Proportions of Conversion. *Note.* IgA = immunoglobulin A; IgG = immunoglobulin G.

In our study, after the peak in SARS-CoV-2 specific human milk IgA following the second dose, a decline was observed. As our sample collection stopped 15 days after the second dose, it is unknown whether SARS-CoV-2 specific human milk antibodies reach a plateau or continue to decline. A recent study by Sterlin et al. (2021) emphasized the importance of IgA in the initial phase of the immune response. Although they did not look at IgA in human milk specifically, this could potentially explain the observed decline in IgA levels in the weeks following the second dose. A randomized controlled trial examining IgA levels in human milk following influenza vaccination found that IgA against the influenza virus remained present for up to 6 months (Schlaudecker et al., 2013). The results of the COVID MILK – POWER MILK study indicated that after natural infection with SARS-CoV-2, IgA antibodies in human milk remain detectable up to at least 10 months post-infection (Juncker et al., 2021). How long SARS-CoV-2 specific antibodies stay present in human milk after vaccination remains to be elucidated.

In serum, we observed a monotonical increase of SARS-CoV-2 specific IgG levels over time. Our findings were consistent with those reported in a BNT162b2 phase I/II trial, showing an increase in SARS-CoV-2 specific serum IgG levels in the first 21 days after the first and second doses of the vaccine (Sahin et al., 2020). Lau et al. (2021) studied the natural antibody response against SARS-CoV-2 in serum and found that even though antibody levels become undetectable over time, the immunological memory leads to a rapid response with higher antibody levels the second time the body is exposed to the antigen. This is in line with our findings following the second dose of BNT162b2, where we measured a faster and steeper increase of milk IgA antibodies over time. Moreover, two of the participants in our study cohort had pre-existing SARS-CoV-2 specific antibodies in their milk and showed a faster response in the increase of SARS-CoV-2 specific IgA.

Although lactating mothers were not included in vaccination trials, they are able to receive the vaccination. To date, due to the lack of knowledge about the effect of vaccination in lactating women, it is difficult to assess the advantages versus the possible risks. It is unlikely that the vaccine lipid is transferred to human milk (Academy of Breastfeeding Medicine, 2020), and indeed it was shown that mRNA-based vaccine compounds were not detected in human milk in the first 4 –48 hr post-vaccination (Golan, Prahl et al., 2021*). Moreover, there is no risk of acquiring infection from the BNT162b2 vaccine (Adhikari & Spong, 2021). Our findings offer insight into the beneficial effect of SARS-CoV-2 vaccination since levels of SARS-CoV-2 specific antibodies were found in human milk after vaccination, which might serve as a protection for infants against COVID-19. Our results empower lactating women to make an informed decision about whether to get vaccinated. As we only followed the participants for 2 weeks after the second dose, future research should focus on the levels of antibodies in human milk over a longer time. The strengths of this study are the timing of sample collection (pre- and post-vaccination), small sampling interval and the standardized way of milk sample collection, enabling a detailed overview of the levels of SARS-CoV-2 specific antibodies following vaccination.

Limitations

One of the limitations of the current study is the short follow-up, which did not allow capturing the levels of SARS-CoV-2 specific IgG and IgA levels beyond 2 weeks after the second dose. Furthermore, the neutralizing capacity of the antibodies was not measured, although this is essential for the protection of the breastfed infant. However, neutralizing capacity of SARS-CoV-2 specific antibodies in human milk has been demonstrated (*van Keulen et al., 2021).

Conclusion

After vaccination with the mRNA-based BNT162b2 vaccine, a SARS-CoV-2 specific antibody response was detected. In human milk, a biphasic response was observed, with SARS-CoV-2 specific IgA starting to increase between Days 5 –7 after the first dose and declining after Day 15, on average. After the second dose, an accelerated immune reaction was observed. The presence of SARS-CoV-2 specific IgA in human milk after vaccination is important as antibodies are transferred via human milk and may serve as a protection for infants against COVID-19. This information can help lactating women in making a well-informed decision about whether to get vaccinated.

*Editor's Note

References marked with * are preprints. Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

**Editor's Note

JHL has a policy of not publishing references from predatory publishers. The references in the Reference List with ** were published in journals whose publishers have been criticized by some academics for low standards of peer review as well as some allegations of academic misconduct. Others have felt these

publishers have done their due diligence. Due to the importance of the topic covered in this review, we left the inclusion of these articles to the authors' discretion. The authors have reviewed all references and take responsibility for their quality.

Disclosures and conflicts of interest

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: JBvG is the founder and director of the Dutch National Human Milk Bank and a member of the National Health Council. JBvG has been a member of the National Breastfeeding Council from March 2010 to March 2020.

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