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Presence of Lactic Acid Bacteria and Nutritional Content in Human Milk in Vaccinated and COVID-19 Infected Mothers

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ABSTRACT

Background: *Breastfeeding provides newborns with the nutrients and biological compounds necessary for their development. The COVID-19 pandemic generated concern in the health sector due to the lack of knowledge of the risk of transmission of SARS-CoV-2 from the mother to her child. It has been shown that human milk does not transmit the virus but rather provides antibodies against SARS-CoV-2.*

Objectives: *The aim was to evaluate the presence of lactic acid bacteria (LAB), and nutritional and immunoglobulin content in the human milk of mothers sick with COVID-19 and vaccinated to determine the effect of the disease and the vaccine applied on breast milk.*

Materials and Methods: *During the pandemic (March–April 2021), human milk samples were collected from breastfeeding mothers who had COVID-19 or were vaccinated. The mothers were divided into three groups: (1) healthy, (2) COVID-19 positive, and (3) vaccinated against SARS-CoV-2. Immunoglobulin levels were measured using ELISA assays with specific kits, and macronutrient concentrations (proteins, lipids, and lactose) along with energy content were analyzed using LACTOSCAN SA (Milplan). Additionally, the content of lactic acid bacteria (LAB) was determined by CFU/mL, and PCR was performed to analyze Lactobacillus gene expression.*

Results: *The results showed that milk from vaccinated mothers had higher fat and lactose content associated with healthy mothers, suggesting that vaccination might affect certain nutritional components of breast milk. Likewise, COVID-19-infected and vaccinated mothers showed higher calorie levels, which could reflect an adaptive metabolic response to the infection. A significant decrease in LAB was also observed in the milk of COVID-19-infected mothers.*

Conclusion: *COVID-19 infection, like maternal vaccination, can influence human milk's nutritional composition and LAB content. Although these variations in composition do not seem to compromise the nutritional quality of milk, they highlight the importance of continuing to investigate the long-term effects of both infection and vaccination on lactation.*

Keywords

Human milk, COVID-19, Nutritional composition, Vaccination, Breastfeeding.

Introduction

Human milk (HM) is an essential food for the newborn, providing nutrients, immunological factors, hormones, and enzymes that contribute to its growth and the maturation of its immune system.

Its nutritional composition includes 87% water, 1% protein, 7% lactose, and 3.8% fat; lactose and fat provide more caloric content [1]. Human milk adapts to the infant's specific needs, going through phases such as colostrum, transitional milk, and mature milk, each with unique bioactive profiles that promote infant health [2].

The composition of human milk varies depending on the nutrition, age, and genetics of the mothers, as well as the stages of lactation (colostrum, transition milk, and mature milk). The protein fraction is the most stable, while the fat content is more variable depending on the different stages of milk maturation, and the mother's diet. The higher the lipid intake from the diet, the higher the fat content of human milk [3,4]. In addition to its nutritional content, human milk plays a crucial role in the immunological protection of the infant, offering lymphocytes, antibodies, and other protective factors from the first days of life [5].

Human milk is key in the initiation and development of the newborn's intestinal microbiota, since this fluid provides a continuous supply of beneficial bacteria during the lactation period, performing anti-infectious functions. Recent studies suggest that at least a significant proportion of the commensal bacteria present in human milk could originate in the mother's intestinal microbiota, reaching the epithelium of the mammary gland through an endogenous route. The species that make up this bacteriome contribute to a homeostatic environment, the alteration of which can modify the structure and function of other microorganisms in the system [6]. It has also been reported that probiotic strains of *Lactobacillus fermentum* CECT5716 and *Lactobacillus salivarius* CECT5713 can exert differentiated immunomodulatory effects. The first strain has immunostimulatory properties, while the second has anti-inflammatory effects, which shows the complexity and impact of human milk on the immune regulation of the newborn [7].

The COVID-19 pandemic generated by the SARS-CoV-2 virus raised concerns about the safety of breastfeeding infants, due to the possible risk of transmission of the virus through breast milk. Subsequently, it was reported that human milk does not transmit the virus, even when the mother is infected, and that human milk maintains its beneficial properties, including the provision of antibodies such as IgA and IgG, specific for neutralizing SARS-CoV-2 [8,9]. It was shown that the consumption of milk extracted from the mammary gland by the newborn does not lead to the transmission of SARS-CoV-2 [9]. This underlines the importance of breastfeeding since it's a source of immunological protection for the infant.

The COVID-19 pandemic has highlighted the need to understand how viral infections and vaccination can affect breastfeeding. In this context, it is essential to investigate and confirm that human milk maintains its nutritional composition during infections such as COVID-19, and after vaccination against SARS-CoV-2. This reaffirms the safety and continued benefits of breastfeeding, underlining its essential role in the health and development of the infant under any circumstances [9]. Likewise, it is very important to determine whether SARS-CoV-2 infection, vaccination against the same virus, or the combination of both, impact the composition of human milk and the content of lactic acid bacteria, to be sure that there are no changes in the composition of human milk, to generate preventive and therapeutic measures according to the results.

Materials and Methods

Study population

The study population consisted of 47 lactating mothers recruited at the "Fray Antonio Alcalde" Civil Hospital and the Milk Bank of the General Hospital of the West (Zoquipan) in the Metropolitan Area of Guadalajara, México. The donors were informed of the study and signed an informed consent. It followed confidentiality under the provisions of the General Health Law on Health Research, Title 5, sole chapter, article 100, Section I to VIII, always taking care of the patient's integrity [23]. Also, the protection of the patient's personal information, as stipulated in the Federal Law on the Protection of Personal Data Held by Private Parties [24]. The inclusion criteria were mothers breastfeeding, who were diagnosed with COVID-19 by PCR test, or were vaccinated against COVID-19. Those mothers who did not produce sufficient milk (<2 mL per breast), as well as mothers with infections such as HIV or syphilis, were excluded. Mature milk samples were collected between 5 and 15 days after COVID-19 diagnosis or vaccination, for 6 months in 2021. Donors followed a standardized hygiene protocol before extraction, which included hand washing and disinfection of extraction equipment, according to the recommendations of the Pan American Health Organization [10]. The samples were stored in sterile tubes and transferred to the laboratory under controlled temperature conditions for subsequent analysis.

Study Design

The type of study is non-probabilistic, consecutive, and convenient. An observational, cross-sectional, and analytical study was carried out with the bromatological analyses of human milk, obtained from 47 mothers distributed in groups: 1) healthy mothers, 2) sick with COVID-19, and 3) vaccinated against SARS-CoV-2.

Nutritional Analysis

Nutritional analysis of human milk samples was performed using a LACTOSCAN SA MILK AN-ALYZER® (Milplan). This equipment measured the total composition regarding fat content, non-fat solids (NFS), protein, lactose, water content, pH, freezing point, energetic content, and density. For this purpose, 10 mL of milk per sample was analyzed, assembling the test tubes directly to the equipment through a supply pump. The analysis was completed in 10 minutes per sample.

Determination of Immunoglobulins

The Quantification of human milk immunoglobulins M (IgM), G (IgG), and A (IgA) was performed by ELISA assays using specific kits: Human IgM ELISA Kit, Human IgG ELISA Kit, and Human IgA ELISA Kit, all purchased from Thermo Fisher Scientific (USA). These kits are designed on the principle of sandwich immunoassay and provide the necessary reagents, including plates sensitized with specific antibodies, diluents, wash solutions, and

chromogenic substrate. Samples, controls, and standards were processed following the manufacturer's instructions. Absorbance readings were performed at 450 nm using a Thermofisher Multiskan Go microplate reader. Immunoglobulin concentrations were determined by interpolation on standard curves generated with the standards included in each kit.

Isolation, Identification, and Gene Expression Analysis of LAB in Human Milk

The quantification of LAB in human milk samples was carried out using the pour plate technique, following ISO 15214:1998, with MRS agar incubated under anaerobic conditions at 37°C for 48 hours. After purification, the identification of isolates was performed using the VITEK® 2 Compact system, specifically targeting *Lactobacillus* species. For confirmed *Lactobacillus* spp., gene expression analysis was performed by extracting total RNA, synthesizing cDNA, and performing qPCR using validated species-specific primers for 37 species of interest. Expression levels were normalized to the 16S rRNA gene and analyzed using the 2^(-ΔΔCt) method.

Statistical Analysis

A comprehensive statistical analysis was conducted to evaluate the effects of COVID-19 infection and SARS-CoV-2 vaccination on the macronutrient composition and the presence of LAB in human milk. Results from these groups were compared to a control group consisting of healthy mothers with no history of COVID-19 infection and no prior COVID-19 vaccination at the time of sample collection. Descriptive statistics were calculated to determine central tendency measures and dispersion measures for bromatological and microbiological variables. Data normality was assessed using the Shapiro-Wilk test, confirming normal distributions across all groups. Comparisons between groups (mothers with prior COVID-19 infection, vaccinated mothers, and healthy controls) were performed using analysis of variance (ANOVA) for independent samples. In cases where statistically significant differences were detected ($p<0.05$), post hoc Tukey and Bonferroni tests were applied to identify pairwise differences between groups. All statistical analyses were conducted using JASP software version 0.19.0.0 and Statgraphics Centurion, applying additional tests required based on the distributional characteristics and type of variable analyzed.

Results

Nutritional Content in Human Milk

The macronutrient composition and caloric content of human milk were compared across three groups: mothers with a history of COVID-19 infection, vaccinated mothers, and healthy controls, Figure 1. Protein concentrations, Figure 1a, were slightly higher in the milk of mothers with a history of COVID-19 infection compared to healthy controls, while vaccinated mothers showed intermediate values. Protein shows levels varied slightly between groups. Mothers with a positive diagnosis of COVID-19 (1.22 ± 0.069 g/dL), those vaccinated against COVID-19 (1.16 ± 0.11 g/dL) and healthy mothers (1.09 ± 0.11 g/dL) had higher protein content. The differences were statistically significant, with an ANOVA analysis showing F= 5.06 and a p-value = 0.0115. There is a statistically significant difference between the means of the three variables at a 95.0% confidence level.

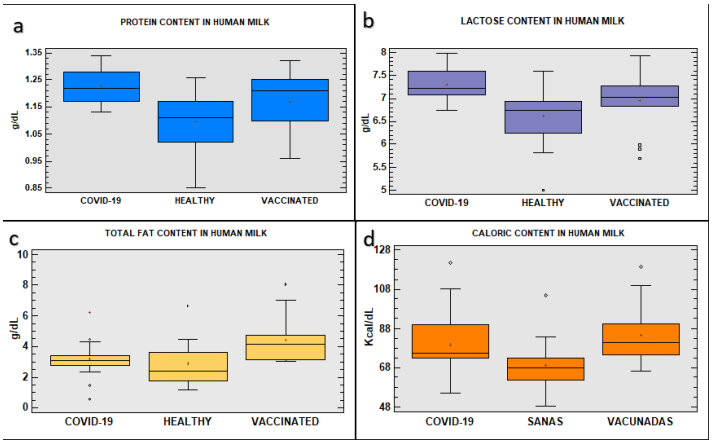

Figure 1: Macronutrient content in the analyzed groups of human milk. In Figure A), the protein content in each of the groups is shown with a p-value of 0.0115. In Figure B), the variations in each of the groups concerning lactose are shown (p=0.0268). In figure c), the values for total fats are displayed (p=0.0368), and in figure d), the values for caloric content are shown (p=0.0267).

ANOVA revealed a significant difference in lactose content among the three groups (F =4.01, p=0.0268). As shown in Figure 1b, mothers with a positive COVID-19 diagnosis exhibited the highest lactose concentration (7.29 ± 0.35 g/dL), followed by those with a history of COVID-19 who were later vaccinated (6.95 ± 0.71 g/dL) and, finally, healthy mothers (6.60 ± 0.66 g/dL). Given that the p-value from the F-test was below 0.05, these differences are considered statistically significant at a 95% confidence level. Regarding lipid content (Figure 1c), an increasing trend was observed in mothers with COVID-19; however, the statistical analysis did not detect a significant difference (p = 0.23). The highest lipid concentrations were found in mothers with a positive COVID-19 diagnosis (4.816 ± 1.812 g/100 mL), followed by vaccinated mothers (3.679 ± 2.291 g/100 mL), those with a history of COVID-19 who were later vaccinated (3.169 ± 1.585 g/100 mL), and lastly, uninfected and unvaccinated mothers (2.887 ± 1.498 g/100 mL).

Statistically significant differences were also observed in the caloric content of human milk (p = 0.0427, ANOVA), as presented in Figure 1d. On average, vaccinated mothers had the highest caloric content (84.23 ± 14.65 Kcal/dL), followed by mothers with prior COVID-19 infection (79.68 ± 17.77 Kcal/dL), and finally, healthy mothers (69.35 ± 13.51 Kcal/dL). The highest intragroup variability, expressed as the coefficient of variation (CV %), was observed in the COVID-19 group (22.30%), followed by the healthy group (19.48%) and the vaccinated group (17.40%). These variations might reflect individual differences in metabolic responses related to infection or vaccination, these findings suggest that both SARS-CoV-2 infection and vaccination may induce immunometabolic adaptations in lactating mothers, leading to an increase in the caloric content of breast milk. This response could serve as a compensatory mechanism to maintain

an adequate energy supply for the infant under immunologically or inflammatory conditions.

SARS-CoV-2 infection has been associated with significant differences in the composition of 67 proteins, 385 lipids, and 13 metabolites in breast milk. A change in 8 proteins was observed in the milk of mothers vaccinated with the mRNA-based Moderna after 6 h of administration, 4 changed proteins were observed for the mRNA-based Pfizer vaccine, and 13 changed proteins were observed with the adenovirus-based Johnson and Johnson vaccine, both after 3 days of administration. The proteins that changed after natural infection and the Johnson and Johnson vaccine were mainly associated with systemic inflammatory responses. Regarding the lipid content in breast milk, it was associated with changes in 385 molecular lipid species (195 increased and 190 decreased). An increase in 66 triacylglycerol species and a decrease in 61 free fatty acids and 57 diacylglycerols were highlighted. Among glycerophospholipids, phosphatidylethanolamine and lysophosphatidylethanolamine were the most upregulated subclasses with 44 and 19 upregulated lipids, respectively. There was also a reduction in 20 fatty acid esters of hydroxyl fatty acids (generally anti-inflammatory lipids) and an increase in 8 ceremide species (usually considered pro-inflammatory lipids). These results show that SARS-CoV-2 infection likely impacts milk lipid composition, in general, by elevating pro-inflammatory lipids and reducing anti-inflammatory ones [11].

Human Milk Immunoglobulins in Response to COVID-19 and Vaccination

Overall, the immunoglobulin content in human milk samples did not show statistically significant differences ($p>0.05$) between the groups (Figure 2). The analysis of IgA concentrations across the four study groups showed similar median values, around 20 ng/dL, as illustrated in Figure 2a. However, some outliers were identified, particularly in the groups of mothers who had COVID-19, where a few samples showed unusually low IgA levels. A Kruskal-Wallis test was performed to evaluate potential differences between groups, since the data did not follow a normal distribution. This analysis revealed a statistically significant difference between the groups ($H=10.42$, $p=0.0055$).

Pairwise comparisons were then conducted using Dunn's test with Bonferroni adjustment for multiple comparisons. A significant difference was observed between the healthy and vaccinated groups ($U=33.0$, adjusted $p=0.0091$). In contrast, the comparisons between healthy and COVID-19 groups ($U=148.0$, adjusted $p=0.0688$), as well as between vaccinated and COVID-19 groups ($U=73.0$, adjusted $p=0.7809$), did not show statistically significant differences after correction. These findings suggest that vaccination may be associated with higher IgA concentrations in human milk related to healthy mothers. On the other hand, COVID-19 infection did not significantly affect IgA levels in comparison to the other groups. However, further studies with larger sample sizes would be needed to confirm these results. Regarding IgM concentrations, the Kruskal-Wallis test showed clear differences between the groups ($H=29.89$, $p=0.000000323$). According to Dunn's post hoc test with Bonferroni correction, mothers who had COVID-19 had significantly higher IgM concentrations compared to both healthy mothers (adjusted $p=0.000012$) and vaccinated mothers (adjusted $p=0.000011$). No statistically significant difference was found between the healthy and vaccinated groups (adjusted $p=0.4754$). These results suggest that COVID-19 infection may lead to an increase in IgM levels in human milk, while vaccination does not appear to influence IgM concentrations when compared to healthy mothers.

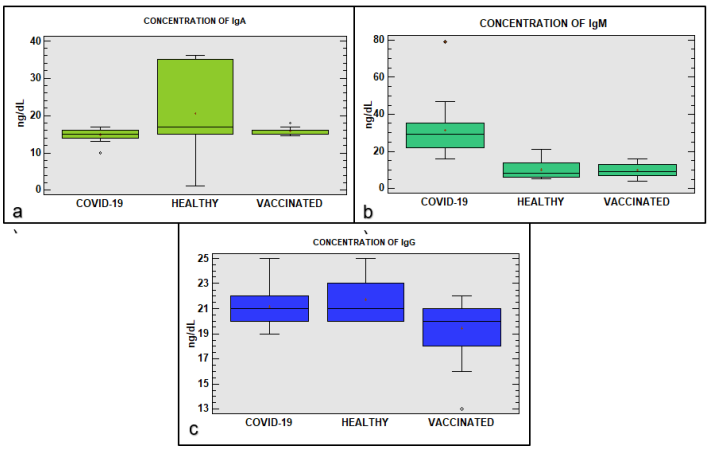

Figure 2: Concentrations of immunoglobulins (IgA, IgM, and IgG) in human milk across different groups. Boxplots represent the distribution of IgA (Figure 2a), IgM (Figure 2b), and IgG (Figure 2c) levels in mothers with COVID-19, healthy mothers, and vaccinated mothers.

The Kruskal-Wallis test revealed statistically significant differences in IgG concentrations among the groups ($H=17.79$, $p=0.00014$). Post hoc comparisons using Dunn's test with Bonferroni correction indicated that vaccinated mothers had significantly lower IgG levels compared to COVID-19 (adjusted $p=0.0039$) and healthy mothers (adjusted $p=0.0004$), while no significant differences were observed between COVID-19 and healthy groups (adjusted $p=0.7879$). These results, illustrated in Figure 2c, suggest that vaccination may be associated with a reduction in IgG levels in human milk, whereas COVID-19 infection does not appear to significantly alter this immunoglobulin compared to healthy individuals.

It has been demonstrated that vaccination against COVID-19 in breastfeeding mothers can protect the mother and her breastfed baby through breast milk [12]. It is also mentioned that maternal vaccination during the lactation stage, with an mRNA-based vaccine, results in a greater antibody response against SARS-CoV-2 in human milk, making this vaccine the best option for mothers and their infants [13]. A study on human milk samples from 86 lactating women vaccinated with BioNTech/Pfizer, Moderna-mRNA, and AstraZeneca, showed strong reactivity for IgG and IgA after vaccination, mainly after the second dose. The presence and persistence of SARS-CoV-2-specific antibodies in breast milk depended on the type of vaccine, with higher levels of IgG and IgA in mRNA-based vaccines compared to AstraZeneca, and on previous exposure to the virus. A high variability of antibodies was observed in the milk of vaccinated women, regarding non-

vaccinated or infected with COVID-19. Anti-SARS-CoV-2 IgG levels were significantly higher, while IgA levels were lower than in the milk of women infected with COVID-19 [14].

Quantification of Specific Antibodies in Vaccinated Breastfeeding Mothers

To validate the detection method for specific antibodies in human milk, an Enzyme-Linked Immunosorbent Assay (ELISA) was performed to quantify three key markers: Nucleocapsid (N), Receptor Binding Domain (RBD), and S1 subunit of the Spike protein. In the negative control, no detectable levels of N, RBD, or S1 were observed, confirming the absence of prior exposure to SARS-CoV-2 and establishing the lower detection limit of the assay. Conversely, positive control exhibited strong reactivity, with antibody levels of 80 for N, 100 for RBD, and 100 for S1, indicating a robust immune response. These results confirm the sensitivity and specificity of the ELISA-based quantification method, ensuring reliable detection of vaccine-induced antibodies in the subsequent analysis.

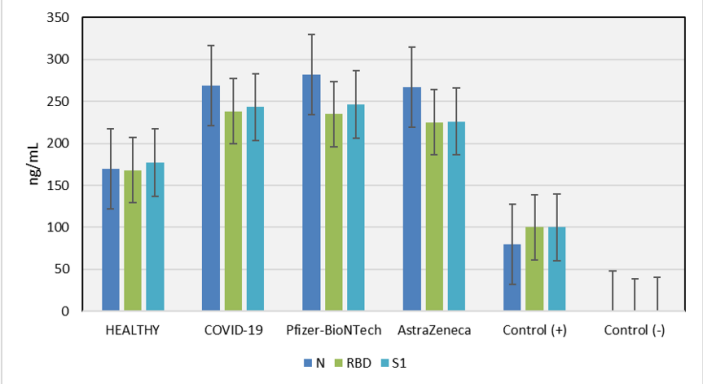

Figure 3: Comparison of SARS-CoV-2-specific antibody levels in human milk among study groups

To analyze differences in the concentration of SARS-CoV-2-specific antibodies in human milk, the levels of Nucleocapsid (N), Receptor Binding Domain (RBD), and the S1 subunit were compared across study groups (mothers with COVID-19, those vaccinated with Pfizer-BioNTech, those vaccinated with AstraZeneca, and the healthy control group). First, a Kruskal-Wallis test was performed, revealing no statistically significant differences among the groups ($p>0.05$ for all markers). Since the data did not follow a normal distribution, Dunn's post hoc test with Bonferroni correction was applied for pairwise comparisons, which confirmed that no significant differences were found in N, RBD, or S1 levels between the evaluated groups (adjusted $p>0.05$). These findings suggest that neither COVID-19 infection nor vaccination with Pfizer-BioNTech or AstraZeneca leads to substantial differences in antibody levels in human milk. However, individual variability in immune response should be considered, along with other factors that may influence the transfer of antibodies to human milk.

LAB content determination

The results obtained from the microbiological analysis of human milk to identify Lactic Acid Bacteria (LAB) are shown in Figure 4. We observed a significant decrease ($p=0.001$) of these bacteria in the milk of mothers who had COVID-19, compared to the milk of healthy mothers who did not get sick with COVID-19 and were not given the vaccine. It is likely that medications, as well as some chronic non-communicable diseases of the mothers, together with the symptoms of COVID-19, caused the decrease in the content of LAB during the disease. Although, in the milk of women who did not get sick and were vaccinated, the content of LAB was present in the milk, between $8x10^1$ and $1.2x10^3$ CFU/mL. It is important to note that in human milk from mothers who had COVID-19, where LAB growth was identified, they were asymptomatic mothers during the COVID-19 infection.

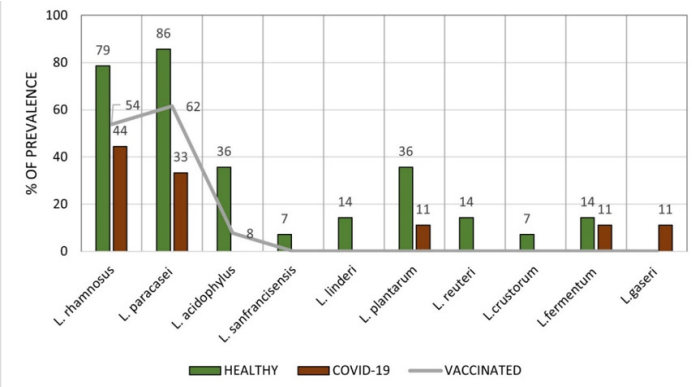

Figure 4: LAB content in human milk in lactating mothers.

In the human milk of COVID-19 positive mothers, we did not find any growth of LAB. We believe that may be due to the consumption of medications against SAR-CoV2, or possibly it is due to the immune system that at that time was too active against the mother's illness, eliminating the bacteria present in the milk of the mothers. A study of the fecal microbiota in hospitalized COVID-19 patients showed significant alterations in the microbiota in relation to disease severity [15]. Symbiotic bacteria were significantly reduced, while there was an increase in opportunistic flora. Different commensal genera of the Firmicutes phylum (Eubacteriaceae, Ruminococcaceae, and Lachnospiraceae) were reduced in patients with COVID-19. Symbiotic bacteria, F. prausnitzii spp. and A. onderdonkii spp. were the main species that showed a negative correlation with COVID-19 severity. The results of this study indicated that the immune response to COVID-19, and consequently the severity of the infection, could be influenced by the alteration of the bacterial flora that usually affects these patients [15,16].

These results are the first finding that demonstrates the decrease in LAB during infection caused by COVID-19; therefore, it is important to highlight the fact that in the milk of healthy but vaccinated women, there is a higher amount of LAB, while in the milk of those who became ill with COVID-19, there was a considerable decrease in LAB. However, it is too early to generate a hypothesis, since it is known that in the face of a viral infection, the cytokines that increase the most are interferons, which may be involved in the decrease in lactic acid bacteria. Likewise, the mother's diet may be a factor that benefits or affects this

decrease in LAB. Alemán Duarte et al. [17], report that in obesity, arterial hypertension, and diabetes mellitus in lactating women, a significant reduction in *Lactobacillus* is observed in human milk.

Discussion

COVID-19 vaccination appears to induce a differentiated immune response in lactating mothers, particularly in the concentration of IgM in human milk. This pattern may be because the COVID-19 vaccine is designed to induce a protective immune response, mainly emphasizing humoral and cellular immunity through the production of virus-specific IgG antibodies [18]. However, the absence of a differential effect on IgA and IgG in the samples suggests that vaccination does not substantially impact the basal concentrations of these immunoglobulins in human milk, which could be modulated by independent factors, such as lactation physiology or passive transfer of immunity [19]. Furthermore, the results show that there were no significant differences in any comparison of IgA and IgG between the groups studied, reinforcing the hypothesis that these immunoglobulins maintain a homeostatic level in breast milk, possibly due to their physiological regulation to ensure the infant's immune protection.

The observed difference in IgM production could be interpreted as an early or transient response to the vaccine antigenic stimulus in the context of local immunity of the mammary gland. Although IgM is not usually predominant in mucosal secretions such as human milk, its increase in vaccinated mothers could reflect a more pronounced localized immune activation, which could benefit the infant by offering a first line of defense against viral infections [20]. In contrast, in mothers with a previous diagnosis of COVID-19, IgM production could be related to an immune response induced by active or recent infection, implying a differential reinforcement based on the interaction between previous infection and vaccination.

Selam-Royo et al. [14]. collected 582 human milk samples from 86 lactating women vaccinated with BioNTech/Pfizer (BNT162b2, n=34), Moderna (mRNA-1273, n=20), and AstraZeneca (ChAdOx1 nCoV-19, n=32). Higher IgG and IgA content was observed after vaccination. In the milk of vaccinated women, anti-SARS-CoV-2 IgG levels were significantly higher, while IgA levels were lower than in the milk of women infected with COVID-19. Women with previous COVID-19 increased their IgG antibody levels after the first dose to a similar level observed in vaccinated women after the second dose. The presence of antibodies against SARS-CoV-2 in breast milk will depend on the type of vaccine that the mother has been administered, finding higher levels of IgG and IgA in the milk of mothers vaccinated with the mRNA vaccine, compared to mothers infected by the virus and those vaccinated with AstraZeneca.

Comparisons between groups revealed that vaccinated mothers (VM) produce significantly higher IgM than unvaccinated mothers (DPC-19 and SINV). This suggests that vaccination is a key modulator of humoral immunity in human milk. However, the absence of significant differences between VC-19 and VCC-19 indicates that prior diagnosis of COVID-19 does not substantially modify the vaccine-induced response. Furthermore, the small differences between DPC-19 and SINV reinforce that prior infection without vaccination does not generate a sustained increase in IgM in human milk. These results could be due to various factors, such as the time elapsed from vaccination or infection to sample collection, the type of vaccine administered (mRNA, viral vector, etc.), or the physiological condition of the mothers, including lactation status, stress and nutrition [21]. On the other hand, the stability of the concentration of IgG and IgA in breast milk could be due to a homeostatic phenomenon where immunoglobulins are regulated independently of the vaccine stimulus, to ensure a stable composition of protective immune factors transferred to the infant.

According to Amezcua-López et al. [22], the content of lactic bacteria in human milk may vary depending on the health condition of the mothers, as well as the consumption of anti-nutritional compounds such as drugs of abuse, medications, and alcohol, among other factors. In our study, LAB was reduced in the milk of mothers infected with COVID-19, but a considerable reduction was also observed in mothers who had COVID-19 and were also vaccinated. This reduction could be determined by the possible antibiotic treatments to which they were subjected. Many factors have been identified that contribute to the variability of bacteria in LH due to various physiological, hormonal, and pathological conditions [6]. These results are the first finding that demonstrates the decrease in LAB during infection caused by COVID-19; therefore, it is important to highlight that a greater amount of LAB is found in the milk of healthy and vaccinated women, while the milk of mothers who became ill with COVID-19 had a considerable decrease in LAB (Figure 8). We cannot yet generate a hypothesis, since it is known that in the face of a viral infection, the cytokines that increase the most are interferons, which may be involved in decreasing lactic acid bacteria. Likewise, the mother's diet may be a factor that benefits or affects this decrease in LAB.

Conclusion

These findings have important clinical implications, as they suggest that vaccination in lactating women not only protects the mother, but also confers immunological benefits to the infant through breast milk by transferring immunoglobulins. Although variations in protein, lipid, and lactose composition were observed in COVID-19-infected mothers, breast milk remains an important food in the diet of newborns. To better understand the mechanisms of IgM transfer, changes in nutritional composition, and decreased LAB content in the milk of COVID-19-infected and/or vaccinated lactating mothers, further studies involving how these components may vary over time or between different types of existing vaccines are required. However, the lack of a significant effect of prior COVID-19 diagnosis highlights the importance of vaccination as a primary strategy to promote humoral immunity in this context.

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