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Human Milk Immunity and Breastfeeding among Lactating Individuals with COVID-19: A Systematic Review

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ABSTRACT

Safety of infants nursed by their infected mothers is crucial to knowing whether the virus can be transmitted vertically through mothers' milk. This study aims to collect and compile data from available studies on the immunological and microbiological components that can be found in COVID-19 infected mothers who breastfed their infants and to investigate the state of the infants who received breast milk while their mother is still confirmed to be positive with the virus. A systematic review was done from 28 articles that have passed the inclusion and exclusion criteria for the study. Qualitative data synthesis was applied to extract and analyze the results. Breast milk of mothers who were still infected with the virus does not harm the infants and instead giving them the extra protection against them. The contents in the mother's milk also were found to have neutralizing ability against the infectivity of SARS-CoV-2 and able to inhibit neutralizing antibodies (NAb) against receptor-binding domain (RBD) protein mutants. Infants who were infected with COVID-19 did not obtain the virus from their mother's milk and no further complications were found in the infants after they were healed from the virus. Mothers can be well assured to continue breastfeeding their infants while contracting the disease as there is no evidence of virus transmitted through breast milk found. Appropriate strategies and guidelines must be followed during the breastfeeding process to minimize the risk of transferring the virus to infants through other method such as coughing or sneezing.

1. Introduction

Breast milk is commonly known as the best source of food for infants as they promote infant's health and development of their immune system [1]. Some of the fundamental nutrients like carbohydrates, lipids, proteins, vitamins, immunological cells, digestive enzymes, and other bioactive components can be found abundantly rich in human milk. Breast milk is primarily made up of fats, which provide energy and aid in the development of the infant's central nervous system. In addition to nutrients, mother's milk contains non-nutritive bioactive substances such as macrophages, stem cells, and growth factors [2]. For example, stem cells will aid in the development and healing of their

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organs. To add more, because they are in liquid form, they can be easily digested by the undeveloped intestines of newborn infants [3]. Undoubtedly, human milk is truly a biological evolution masterpiece that provides numerous benefits to both infants and new mothers.

Coronavirus disease 2019 (COVID-19) which has been overtaking the globe since early 2020 is a highly contagious disease outbreak caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a virus from the Coronaviridae family [4]. This is a respiratory condition that is especially fatal in vulnerable populations including the elderly and those with a history of sickness. Breastfeeding moms and their newborns are among the most vulnerable members of the high-risk community to the disease [5].

Although COVID-19 vaccines have been developed, there remains uncertainty about whether SARS-CoV-2 can be transmitted via human milk. This is a critical gap, as addressing it may help develop recommendations for breastfeeding practices among COVID-19-positive mothers. Besides, given that infants lack the capability to produce their own antibodies and rely heavily on human milk as their primary source of nourishment, it is essential to investigate if human milk provides any protective factors or if it poses a risk of viral transmission. This gap clearly highlights the need for research on the immunological properties of human milk in the context of COVID-19. Despite significant research on the impact of COVID-19 on various demographics, there remains limited information on the impact on breastfeeding mothers and their infants. This gap underscores the necessity for targeted studies to ensure informed recommendations for this particularly vulnerable group.

Based on the research gaps, this study will utilize the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol (PRISMA-P) 2020 [6] as a guideline. By adhering to the PRISMA guideline, the rigor and transparency of the systematic review process is enhanced. Moreover, this approach supports a comprehensive and unbiased synthesis of findings, making the conclusions more reliable and potentially impactful for guiding healthcare practices regarding breastfeeding during COVID-19. Hence, this systematic review aims to collect and compile data from available studies on the immunological and microbiological components that can be found in COVID-19 infected mothers who breastfed their infants and to investigate the state of the infants who received breast milk while their mother is still confirmed to be positive with the virus.

2. Methodology

2.1 Systematic Identification of Relevant Studies

Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol (PRISMA-P) 2020 [6] was used as a guideline. The selection procedure is as illustrated in Figure 1**.** Articles were located and gathered utilizing the accessible online databases namely Science Direct, Scopus, and PubMed. The papers were thoroughly and comprehensively filtered using keywords of “human milk” or “breast milk”, that were used interchangeably, while keywords of “immune*” or “antibody”, and “COVID-19” with “SARS-CoV-2” were used alternatively. Other keyword includes “breastfeeding”. The keywords are used together with BOOLEAN operators such as AND or OR enclosed in brackets. The articles were searched by the following keywords: Human milk, OR breast milk AND immune*, OR antibody AND breastfeeding AND COVID-19, OR SARS-CoV-2.

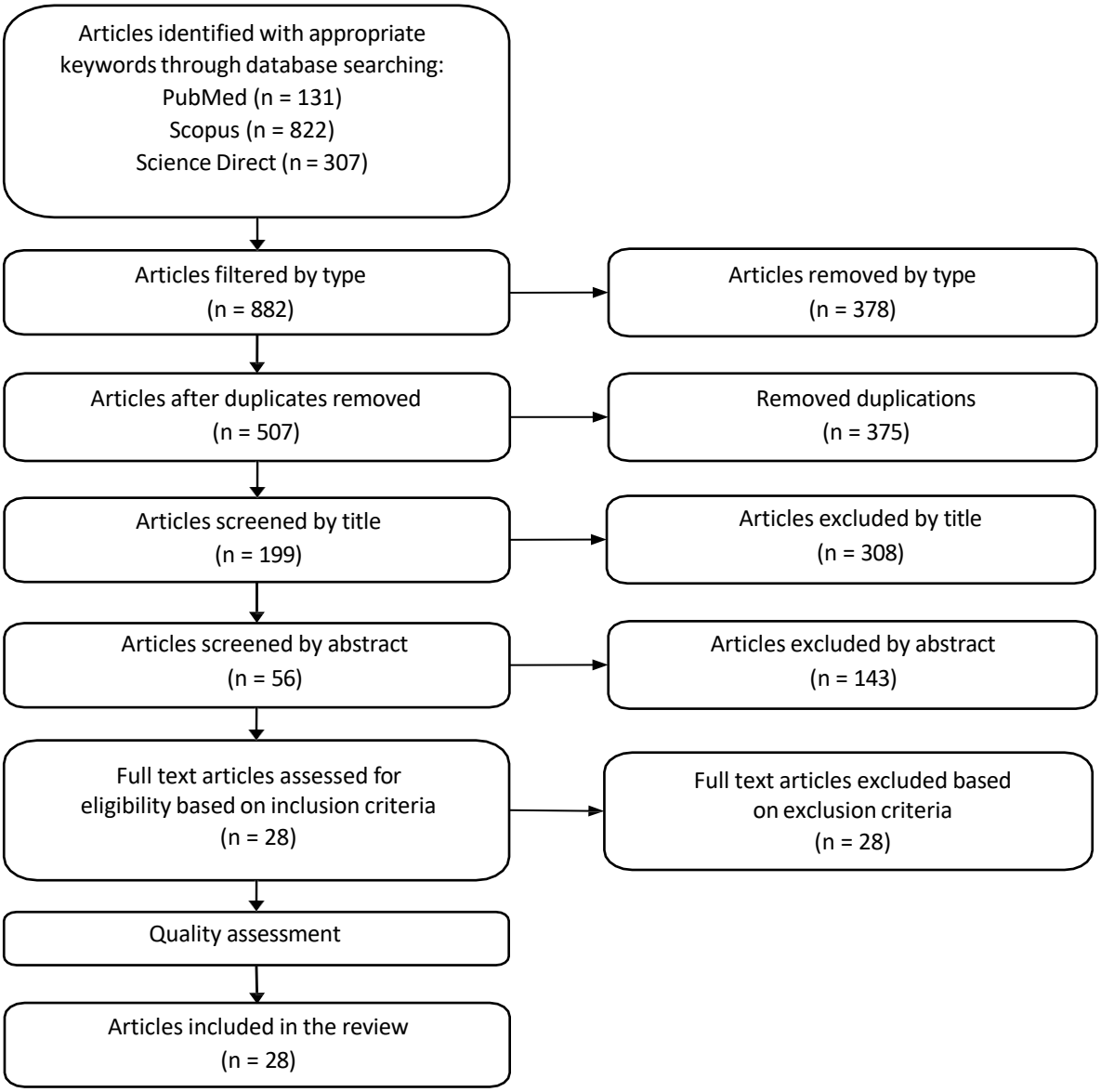

Fig. 1. Flow diagram of selection procedure based on PRISMA 2020

2.2 Inclusion and Exclusion Criteria

Studies involved in this study are original articles that were published in English, within the publication period from 2020 onward. Studies that focused on breastfeeding mothers who are positive COVID-19, and children breastfed by positive COVID-19 mothers were included in this study. Other articles published in other language, non-original articles and studies that focused on content of human milk after the vaccination period, involving mothers who are not breastfeeding currently, and papers focusing on non-pregnant women were excluded from this study. Further details can be referred in Table 1.

Table1
Inclusion and exclusion criteria for systematic review

Inclusion criteria	Exclusion criteria
• Study published in English • Study published in 2020 or more • Original article • Breastfeeding mothers who are positive COVID-19 • Children breastfed by mothers who are positive with COVID-19	• Study published in other languages • Study published before 2020 • Non-original articles (book chapters, review articles, letters) • Studies specifically on content of human's milk after vaccination process • Pregnant mothers who are currently not breastfeeding • Mother who are not breastfeeding • Non-pregnant women • Children who are breastfed by mothers who are not exposed to/positive with COVID-19

2.3 Interrater Reliability

Interrater reliability is used in systematic reviews to evaluate the agreement between all reviewers during the data extraction process. Two reviewers screened the studies for inclusion in this systematic review while referring to the inclusion and exclusion criteria (Table 1), with the main reviewer screening the records for inclusion and the other verifying all decisions. This study uses percent agreement [7] to measure the interrater reliability.

2.4 Quality Assessment

The selected articles were assessed for its quality and eligibility to be included according to Crowe Critical Appraisal Tool (CCAT) [8] version 1.4 checklist of questions. The CCAT consists of the CCAT Form (the Form) and the CCAT User Guide (the User Guide) [8]. The Form and the User Guide were used together to avoid any compromise in the obtained validity and reliability of the scores. The result checklist consists of eight categories: preliminaries, introduction, design, sampling, data collection, ethical matters, results, and discussion that comprises of six-point scale (0, 1, 2, 3, 4, and 5) for each category. The total of these points is divided by 40 to present the total percentage scores of the articles. The percentage is then classified into three groups: high (≥75%), moderate (51% to 74%), or low (≤50%) quality of articles. Articles with more than 70% score were considered as high quality and thus were included in this systematic review.

3. Results

From the 1260 articles found according to the keywords used in database searching. After removing articles that were book chapters, review articles, letters and other irrelevant types, 882 articles were left. Then, 375 articles were removed due to duplications, and another 308 articles were excluded by their titles, leaving 199 articles left. The remaining articles were then screened by abstract, and another 143 were removed due to their abstracts not matched with the aim of this study. The remaining 56 articles were then assessed carefully by reading their full texts and filtered according to the inclusion and exclusion criteria, and a total of 28 articles were removed as they were irrelevant to the target of this research. In total, there were 28 full text papers included in this systematic review after considering their eligibility according to inclusion criteria. The flow of

selecting the articles to be included in this systematic review is shown in Figure 1. The percentage of agreement for selection of articles was 89% (Table 2).

Table 2
Calculation of percent agreement

Study no.	Raters		Difference
	1	2	
1	0	0	0
2	0	0	0
3	0	0	0
4	1	1	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	1	1	0
10	0	0	0
11	0	1	-1
12	0	0	0
13	0	0	0
14	0	0	0
15	0	1	-1
16	1	1	0
17	1	1	0
18	0	0	0
19	0	0	0
20	0	0	0
21	0	1	-1
22	0	0	0
23	0	0	0
24	1	1	0
25	0	0	0
26	0	0	0
27	0	0	0
28	1	1	0
Number of Zeros			25
Number of Items			28
Percent Agreement			89

3.1 Presence of SARS-CoV-2 RNA in Human Milk

Several studies examined and investigated the presence of SARS-CoV-2 RNA in infected mother's milk to deduce whether they can be transferred passively into nursing infants and impact their health. Some researchers have detected SARS-CoV-2 RNA in breast milk, specifically targeting the N1 of the nucleocapsid gene and the envelope (E) gene2, suggesting the presence of viral genetic material [9]. However, other studies have found no trace of the virus in milk samples, indicating a lack of consistent evidence of active virus transmission through breastfeeding [9-19]. Additionally, immune responses play a crucial role. Nevertheless, some studies highlighted that antibodies and immune factors present in the milk may protect infants rather than pose a risk. For instance, maternal antibodies and their neutralizing capacities may serve as passive immunity for infants, helping shield them from infection. This protective element underscores the importance of breastfeeding, even in the context of maternal infection, as these immune factors can bolster an infant's own developing immune system. While SARS-CoV-2 RNA may occasionally appear in breast milk, there is no

conclusive evidence that breastfeeding poses a risk of infection to infants. Instead, breast milk from COVID-19-infected mothers may offer immune benefits, including the transfer of antibodies that could protect infants against the virus. Thus, breastfeeding remains a safe practice, providing essential immune support during early development.

3.2 Expression of Immunological Components Found in Human Milk

Several studies discovered the presence of SARS-CoV-2 specific immunoglobulins (IgA, IgG and IgM) in breast milk [9,11,13,19-27], which play a role in enhancing infants' immune defences. One study found out that human milk IgG were more specific to S2 subunit SARS-CoV-2 than other antibodies [28] while another study uncovered that the IgA in the COVID-19 recovering participants exhibited neutralization of Spike-pseudo-typed VSV [22], potentially providing passive immunity to nursing infants. The amount of SARS-CoV-2 specific IgA was found out to be similar after COVID-19 vaccination and getting infected, although the variability in IgA levels were certainly higher after one was infected compared to vaccinated [29]. The antibodies were found to be remaining in human milk for up to five months after the onset of COVID-19 symptoms [30], and in another study by the same author, they found that the antibodies will remain present in human milk for at least 10 months after a polymerase chain confirmed infection [23], maintaining a protective presence that could reduce the risk of viral transmission to infants. The same study also discovered a high prevalence of IgA antibodies against SARS-CoV-2 which can lead to passive immunity protection for breastfed infants against the virus. One study found out that the milk samples were able to neutralize SARS-CoV-2 infectivity in vitro [26], which is supported by another study which discovered that unpasteurized milk with and without these antibodies could neutralize a pseudovirus of SARS-CoV-2 [27]. One research found that human milk which were collected after infection and vaccination exhibited microneutralization activity, with the said activity increasing with time after vaccination [31].

Lactoferrin, cytokines, chemokines, and growth factors help modulate infant immunity. There was a study that focused on the concentration of lactoferrin in human milk and discovered that there is no difference in concentration of lactoferrin between infected mothers and the controls [32]. However, there is a reduction in concentration for symptomatic mothers compared to asymptomatic and healthy controls [32]. In another study, it was recorded that there was an eightfold increase in IFNα+ milk leukocytes, from 1% before SARS-CoV-2 infection to 8% when actively infected [33] and the milk macrophages showed the highest increase in IFNα expression while there is an actual reduction in the dendritic cells, suggesting enhanced immune readiness in the milk of infected mothers. One paper found that the concentrations of cytokines (IFN-γ, IL-1ra, IL-4, IL-6, IL-9, IL-13, and TNF-α) chemokines (eotaxin, IP-10, MIP-1α, and RANTES) and growth factors (FGF, GM-CSF, IL7, and PDGF-BB) were higher in breastmilk of the infected mothers compared with the control group at 1st week postpartum [18]. The controls that were being investigated along with samples comes from different types, namely, pre-pandemic milk samples [9,20,22,28,33], non-infected COVID-19 mothers [32,35], healthy lactating mothers [14,15,26], and pregnant women who were confirmed to be free of SARS-CoV-2 [16,18].

The findings of immunological components in human milk have been analysed and summarized into Table 3.

Table 3
Findings of immunological components in human milk

Author, Year	Findings in Human Milk (according to study)				
	SARS-CoV-2 RNA	IgA	IgG	IgM	Others
Bäuerl, C *et al.,* (2022) [9]	Not detected in milk samples	Detected	Detected	Detected	Main Protease (MPro) domain antibodies detected
Bertino, E *et al.,* (2020) [10]	Only 1 sample detected the presence out of 14 milk samples				
Briana, DD *et al.,* (2022) [32]					• No differences in Lactoferrin concentrations between SARS-CoV-2 mothers and controls • Symptomatic mothers showed lower breast milk Lactoferrin concentrations compared to asymptomatic mothers and healthy controls
Conti, MG *et al.,* (2021) [46]	• 1 case of potential mother-infant vertical virus transmission • 1 case of horizontal virus transmission				
Demers-Mathieu V *et al.,* (2021) [20]		Detected	Found to be more specific to S2 subunit SARS-CoV-2 than other antibodies	Detected	Presence of pre-existing human milk antibodies against S2 subunit SARS- CoV-2
Demers-Mathieu V *et al.,* (2021) [28]		Detected	Detected	Detected	SARS-CoV-2 RBD-specific IgA, IgM, IgG higher in serum sample group compared to human milk group
Demers-Mathieu V *et al.,* (2022) [21]			√, titer of IgG against N501Y found to be higher in COVID-19 vaccine group than no-vaccine group, but comparable to COVID-19 PCR group		Inhibition on Nab against binding of 2 mutant RBD proteins to their receptors higher in COVID-19 vaccine and PCR than pre-pandemic milks
Fox A *et al.,* (2022) [22]		Detected			6 of 8 COVID-19 samples exhibited neutralization of Spike-pseudotyped VSV

Gao X *et al.,* (2020) [11]	Not detected in human milk samples		Detected	Detected	
Juncker HG *et al.,* (2021) [23]		√, variability of IgA levels higher after infection rather than after vaccination			
Juncker HG *et al.,* (2021) [29]		Detected	Detected	Detected	*Antibodies remain present up to 5 months in human milk after onset of COVID- 19 symptoms
Juncker HG *et al.,* (2021) [30]		√, remain present at least 10 months after infection			
Kilic T *et al.,* (2021) [47]	Detected in milk samples from 4 mothers (over 15 mothers with COVID-19)				
Krogstad P *et al.,* (2022) [12]	Not detected in milk samples				
Lebrão CW *et al.,* (2020) [24]		Detected in milk sample			
Liu W *et al.,* (2021) [25]			Detected	Detected	
Luo, Q. *et al.,* (2021) [13]	Negative			Detected, presence of IgM in breast milk correlated with presence of IgM in maternal blood	
Pace RM *et al.,* (2020) [15]	Not detected in milk samples, but found on several breast swabs				
Pace RM *et al.,* (2021) [14]		Detected	Detected		62% of milk samples able to neutralize SARS-CoV-2 infectivity in vitro
Pace RM *et al.,* (2021) [26]	Not detected in milk samples	Detected, persisted for at least 2 months in 77% women			
Peng S *et al.,* (2020) [16]	Negative				
Pullen KM *et al.,* (2021) [34]		Detected	Detected	Detected	*Preferential transfer of IgA and IgM to breast milk, with a selection of IgG

Sánchez García L *et al.,* (2021) [18]	Not found in breast milk samples				• Concentrations of cytokines (IFN-γ, IL-1ra, IL-4, IL-6, IL-9, IL-13, and TNF-α) chemokines (eotaxin, IP-10, MIP-1α, and RANTES) and growth factors (FGF, GM-CSF, IL7, and PDGF-BB) were higher in breastmilk of the study compared with the control group at 1st week postpartum • Severity of disease (symptomatic or asymptomatic infection) did not affect the immunological profile in breast milk
van Keulen BJ *et al.,* (2021) [27]		Detected	Detected	Detected	• Unpasteurized milk with and without these antibodies was found to be capable of neutralizing a pseudo virus of SARS-CoV-2 • No correlation was observed between milk antibody levels and neutralization capacity
Young BE *et al.,* (2022) [19]					• Human milk collected after infection and vaccination exhibited microneutralization activity • Microneutralization activity increased through time in vaccine group only, but found to be higher in infection group vs after first-dose vaccination
Yu Y *et al.,* (2020) [48]	Not detected in breast milk	Detected	Detected	Detected	
Yu JC *et al.,* (2021) [33]					• Eightfold increase in IFNα+ milk leukocytes (1%->8%) • Highest increase of milk macrophages in IFNα expression • T and B lymphocytes showed mild increase • Dendritic cells showed reduction

3.3 Clinical Outcomes of Infants Nursed by SARS-CoV-2 Infected Mothers

There were considerably fewer papers which specifically mentioned the state of nursing newborns compared to those that investigating the components in human milk. Among them, there is a study that discovered significant higher spike-specific salivary IgA antibody levels in infants who received breastmilk during their first 2 months of life [38] while others only recorded the time span for infants to became SARS-CoV-2 negative after getting infected; within 6 weeks of, after 1 month of their age [17], on the seventh day after hospitalized [19]. The rest of the clinical outcome of infants were presented in Table 4.

Table 4
Clinical outcome of infants breastfed by confirmed COVID-19 mothers

	Effects on children breastfed by COVID-19 infected mothers	
Author, year	Condition of the children while breastfeeding during the time their mother was positive COVID-19	Duration of infants contracting the disease
Bertino *et al.* (2020) [10]		6 weeks
Conti *et al.* (2021) [46]	Significant level of salivary IgA level was found in infants	
Gao *et al.* (2020) [11]	No infants developed COVID-19 while breastfeeding	
Kilic *et al.* (2021) [47]	Mild COVID-19 symptoms on infants who tested positive	
Liu *et al.* (2021) [25]	Infant was asymptomatic	
Luo *et al.* (2021) [13]	No infants infected with the virus	
Prasad *et al.* (2021) [17]	Only 2 out of 51 infants getting infected, and all infants were reported to be healthy after one month of breastfeeding with positive COVID-19 mothers	
Yu *et al.* (2020) [48]		One week

3.4 Risk of Bias Assessment

The result of bias risk assessment can be referred to in Table 5, whereby, as seen in the table, all papers scored above 70%, with the lowest scored 75%, and the highest 95%. Therefore, these papers can be considered as having medium to high quality and were all approved to be included in this systematic review.

Table 5

Result of risk of bias assessments of retrieved articles based on Crowe Critical Appraisal Tool (CCAT)

Item descriptors	CCAT score for study no.:													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Preliminaries	5	5	5	5	4	5	5	3	4	3	4	4	5	4
Introduction	5	5	4	4	4	4	4	3	3	4	3	5	5	5
Design	3	5	4	5	3	4	4	5	4	3	3	5	5	4
Sampling	5	4	4	4	4	4	4	4	4	3	4	5	5	4
Data collection	4	5	4	5	4	4	4	4	4	4	4	5	5	4
Ethical matters	5	5	4	4	3	4	4	4	4	4	4	4	4	4
Results	5	4	5	5	5	5	5	4	5	5	4	5	5	5
Discussion	5	5	5	5	5	5	5	4	5	5	5	5	4	5
Total of CCAT score (%)	93	95	88	93	80	88	88	78	83	78	78	95	95	88
Item descriptors	CCAT score for study no.:													
	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Preliminaries	5	3	4	4	3	4	4	5	3	4	4	5	3	4
Introduction	4	4	4	5	4	4	4	5	4	4	5	4	3	4
Design	4	3	4	5	4	5	5	4	4	4	4	4	3	4
Sampling	4	4	4	4	4	5	4	4	3	4	4	4	3	3
Data collection	4	4	5	5	4	4	5	4	4	4	4	4	4	4
Ethical matters	4	4	5	4	4	4	4	5	4	4	4	5	4	4
Results	5	4	4	5	4	5	4	5	4	5	5	4	5	5
Discussion	5	5	5	5	5	5	4	5	4	5	5	5	5	4
Total of CCAT score (%)	88	78	93	93	80	90	85	93	75	85	88	88	75	80

4. Discussion

This systematic review has addressed the research gaps on whether SARS-CoV-2 can be transmitted through breast milk, and whether transmission could potentially occur through breastfeeding and affect infants' health. Overall, this review has highlighted important data from available studies on the immunological and microbiological components that can be found in COVID-19 infected mothers who breastfed their infants. Additionally, this review has brought relevant evidence on the state of the infants who received breast milk while their mother is still confirmed to be positive with the virus.

When a mother is infected with SARS-CoV-2, her body generates specific antibodies, including immunoglobulins IgA, IgG, and IgM, which are transferred to her breast milk. In this review, it was revealed that SARS-CoV-2 RBD-specific immunoglobulins are present in abundance in all breast milk, which in agreement with their role as part of the adaptive immune response that is activated when the body encounters SARS-CoV-2. The virus has four primary structural proteins [31]. The spike protein (S protein) facilitating its entry and fusion into host cells. This spike protein is divided into two subunits, the S1 and S2. The S1 subunit initially binds to ACE2 receptors, which are the host cell receptors, whereas the S2 subunit allows the viral membrane to fuse with the host cells [35]. Since the S2 subunit is more structurally conserved than S1, it is less prone to mutations, making it a promising target for vaccines designed to maintain efficacy across variants [36] and providing a mechanism for passive immunity to the breastfeeding infant [39,25,40,41]. Disruption of the fusion structure important for cell invasion is achieved by neutralization of the virus by antibodies targeting the S2 subunit [36].

The potential for broad neutralization can be enhanced with the aid of IgG antibodies found in breast milk. IgG antibodies have been proven to exhibit specificity for the SARS-CoV-2 S2 subunit, which is structurally conserved across various viral variants [40,42,43]. Spike-specific IgA antibodies were detected in the saliva of infants who received breast milk from infected mothers [15], aligning with evidence that secretory IgA appears in infants' saliva as early as three days after birth [38], likely from maternal milk intake. Moreover, the IgA antibodies in individuals recovered from COVID-19 bound to the S protein has been shown to neutralize a spike-pseudotyped vesicular stomatitis virus (VSV) [22]. This suggests that COVID-19 recovered breastfeeding mothers may help to reduce the risk of colonization of virus in their breastfed infants [9,39,40]. The levels of IgA in these individuals were shown to be higher than those in vaccinated individuals [29], which are mostly because infection-acquired immunity can persist longer in their body [37], with antibodies remaining for up to 5 to 10 months post-symptom onset [30]. Notably, the immunity can last over a year in individuals who have recovered from infection and subsequently received vaccination [37]. On the other hand, the IgM antibodies, which typically responds early during an infection, play role to further supports the immune response of the breastfed infants. This is achieved by enhancement of the neutralization capacity of breast milk against SARS-CoV-2 [36,44].

Interestingly, clinical observations have shown that infants breastfed by COVID-19-positive mothers generally remain healthy, and there is no evidence of virus transmission through breast milk itself. Studies indicate that infants who contracted the virus often exhibit mild or no symptoms at all, which strongly suggest that the immunological support from breast milk has helped in reducing the severity of SARS-CoV-2 infection [10,41,43]. This protective effect emphasizes the importance of breastfeeding, particularly during pandemics, as it can boost the infant's immune response and relieve the impact of SARS-CoV-2 infections on infants. Moreover, the antibodies present in breast milk can persist for continued periods following maternal infection.

For newborns, the concept of passive immunity is critical, as they rely heavily on maternal antibodies due to their immature immune systems. Though temporary, breastfeeding is the best means to provide immediate immunity to the infants. They also benefit from ongoing protection as research has indicated that SARS-CoV-2 antibodies remain detectable in the breast milk of infected mothers for several months, even as maternal immunity wanes [39,40,43]. This prolonged presence of antibodies is highly important in offering the infants a continued defence against SARS-CoV-2 and potentially other viral infections, particularly if the mother is re-exposed to the virus. Infants continue to receive protective factors that can neutralize the virus, thereby preventing the virus from binding to ACE2 receptors which are its main entry points on cells [40,41,43]. This mechanism is particularly given the limited ability of infants to mount a robust their own immune response. This mechanism is vital given the limited ability of infants to mount a robust immune response on their own [10].

Besides antibodies, breast milk also contains various cytokines, immune cells, and bioactive molecules that can contribute to an infant's immunity, highlighting its main role in providing nutritional and immunological support to the infants. Lactoferrin, for example, which is an antimicrobial and antiviral protein that is present in the breast milk, can limit bacterial growth and exhibits direct antiviral effects by binding to the viral particles, thus inhibiting their ability to infect the host cells [9,39,45]. Moreover, there is an increase in interferon-expressing milk leukocytes in symptomatic mothers. This is potentially providing the infants with enhances protection against viral infections [40,41,43].

4.1 Limitations and Suggestions

There were several limitations identified from the review. This systematic review is not designed to find information on the long-term immunological impact on infants and variation in immune responses between vaccinated and naturally infected mothers. Hence, it is recommended to explore how maternal antibodies, when transferred via breast milk, influence infant immunity, infection resistance, and development over time. On the other hand, more research is needed to compare the immune responses in breast milk from mothers who were naturally infected with SARS-CoV-2 versus those who were vaccinated. This could clarify if and how the immune protection passed to infants differs based on the mother's method of immunization. Furthermore, there is limited data on how varying recommendations around breastfeeding for COVID-19-positive mothers have impacted breastfeeding practices, maternal mental health, and infant nutrition. Research assessing these aspects could guide better-informed public health policies and support measures.

5. Conclusion

This systematic review has successfully consolidated current findings and evidence that supports breastfeeding as a safe and beneficial practice for infants' immunity against COVID-19, particularly through the role of specific antibodies and other immune-boosting components in the breast milk. In this review, the presence and function of key immunological components in the breast milk, particularly SARS-CoV-2-specific antibodies are reviewed thoroughly, thus providing a deeper understanding on how these antibodies can potentially protect infants from SARS-CoV-2 infection. Furthermore, this review has emphasized the concept of passive immunity, underscoring the critical role of maternal antibodies in safeguarding infants, who have immature immune systems. By exploring breast milk's ability to neutralize SARS-CoV-2 in vitro, the review presents evidence supporting its antiviral properties. This review has also documented clinical observations showing that breastfed infants of COVID-19-positive mothers remained generally healthy, with mild or no

symptoms when infected. This outcome strengthens the argument for breastfeeding's protective effect even when mothers are COVID-19 positive. On the other hand, findings on the longevity of maternal antibodies which is up to 10 months post-infection offer valuable insight into the continued protective effects of breast milk, highlighting its role in providing prolonged immune support to the breastfed infants. Beyond antibodies, this review has discussed other immune components such as cytokines, leukocytes and lactoferrin, offering an inclusive perspective on the multifaceted immunological benefits of the breast milk.

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References

[1] Lyons, Katríona E., C. Anthony Ryan, Eugene M. Dempsey, R. Paul Ross, and Catherine Stanton. "Breast milk, a source of beneficial microbes and associated benefits for infant health." *Nutrients* 12, no. 4 (2020): 1039. https://doi.org/10.3390/nu12041039

[2] Tome-Carneiro, Joao, Noelia Fernandez-Alonso, Cristina Tomas-Zapico, Francesco Visioli, Eduardo Iglesias-Gutierrez, and Alberto Davalos. "Breast milk microRNAs harsh journey towards potential effects in infant development and maturation. Lipid encapsulation can help." *Pharmacological research* 132 (2018): 21-32. https://doi.org/10.1016/j.phrs.2018.04.003

[3] Urbaniak, Camilla, Jeremy P. Burton, and Gregor Reid. "Breast, milk and microbes: a complex relationship that does not end with lactation." *Women's Health* 8, no. 4 (2012): 385-398. https://doi.org/10.2217/WHE.12.23

[4] Lai, Chih-Cheng, Tzu-Ping Shih, Wen-Chien Ko, Hung-Jen Tang, and Po-Ren Hsueh. "Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges." *International journal of antimicrobial agents* 55, no. 3 (2020): 105924. https://doi.org/10.1016/j.ijantimicag.2020.105924

[5] Mirbeyk, Mona, Amene Saghazadeh, and Nima Rezaei. "A systematic review of pregnant women with COVID-19 and their neonates." *Archives of gynecology and obstetrics* 304 (2021): 5-38. https://doi.org/10.1007/s00404-021-06049-z

[6] Page, Matthew J., Joanne E. McKenzie, Patrick M. Bossuyt, Isabelle Boutron, Tammy C. Hoffmann, Cynthia D. Mulrow, Larissa Shamseer et al. "The PRISMA 2020 statement: an updated guideline for reporting systematic reviews." *bmj* 372 (2021).

[7] McHugh, Mary L. "Interrater reliability: the kappa statistic." *Biochemia medica* 22, no. 3 (2012): 276-282. https://doi.org/10.11613/BM.2012.031

[8] Crowe, Michael. "Crowe critical appraisal tool (CCAT) user guide." *Conchra House* 10 (2013).

[9] Bäuerl, Christine, Walter Randazzo, Gloria Sánchez, Marta Selma-Royo, Elia García Verdevio, Laura Martínez, Anna Parra-Llorca et al. "SARS-CoV-2 RNA and antibody detection in breast milk from a prospective multicentre study in Spain." *Archives of Disease in Childhood-Fetal and Neonatal Edition* 107, no. 2 (2022): 216-221. https://doi.org/10.1136/archdischild-2021-322463

[10] Bertino, Enrico, Guido Eugenio Moro, Giuseppe De Renzi, Giuseppina Viberti, Rossana Cavallo, Alessandra Coscia, Carlotta Rubino et al. "Detection of SARS-CoV-2 in milk from COVID-19 positive mothers and follow-up of their infants." Frontiers in pediatrics 8 (2020): 597699. https://doi.org/10.3389/fped.2020.597699

[11] Gao, Xuan, Shaoshuai Wang, Wanjiang Zeng, Suhua Chen, Jianli Wu, Xingguang Lin, Yanyan Liu, Ziyong Sun, and Ling Feng. "Clinical and immunologic features among COVID-19–affected mother–infant pairs: antibodies to SARS-CoV-2 detected in breast milk." *New microbes and new infections* 37 (2020): 100752. https://doi.org/10.1016/j.nmni.2020.100752

[12] Krogstad, Paul, Deisy Contreras, Hwee Ng, Nicole Tobin, Christina D. Chambers, Kerri Bertrand, Lars Bode, and Grace M. Aldrovandi. "No infectious SARS-CoV-2 in breast milk from a cohort of 110 lactating women." *Pediatric research* 92, no. 4 (2022): 1140-1145. https://doi.org/10.1038/s41390-021-01902-y

[13] Luo, Qing-Qing, Lin Xia, Du-Juan Yao, Min Wu, Hong-Bo Wang, Min-Hua Luo, Xuan Jiang, and Hui Chen. "Breastfeeding in mothers with COVID-19: insights from laboratory tests and follow-up from early outbreak of the

pandemic in China." *Journal of women's health* 30, no. 11 (2021): 1546-1555. https://doi.org/10.1089/jwh.2020.8978

[14] Pace, Ryan M., Janet E. Williams, Kirsi M. Järvinen, Mandy B. Belfort, Christina DW Pace, Kimberly A. Lackey, Alexandra C. Gogel et al. "Characterization of SARS-CoV-2 RNA, antibodies, and neutralizing capacity in milk produced by women with COVID-19." *MBio* 12, no. 1 (2021): 10-1128. https://doi.org/10.1128/mBio.03192-20

[15] Pace, Ryan M., Janet E. Williams, Kirsi M. Järvinen, Mandy B. Belfort, Christina DW Pace, Kimberly A. Lackey, Alexandra C. Gogel et al. "COVID-19 and human milk: SARS-CoV-2, antibodies, and neutralizing capacity." *MedRxiv* (2020).

[16] Peng, Sicong, Huaping Zhu, Lixia Yang, Li Cao, Xiaona Huang, Michelle Dynes, Anuradha Narayan et al. "A study of breastfeeding practices, SARS-CoV-2 and its antibodies in the breast milk of mothers confirmed with COVID-19." *The Lancet Regional Health–Western Pacific* 4 (2020). https://doi.org/10.1016/j.lanwpc.2020.100045

[17] Prasad, Arun, N. Yankappa, Pradeep Kumar, Bhavesh Kant Chaudhary, Binod Kumar Pati, Monika Anant, and Lokesh Kumar Tiwari. "Excretion of SARS-CoV-2 in breast milk: a single-centre observational study." *BMJ Paediatrics Open* 5, no. 1 (2021). https://doi.org/10.1136/bmjpo-2021-001087

[18] Sanchez Garcia, Laura, Natalia Gómez-Torres, Fernando Cabañas, Raquel González-Sánchez, Manuela López-Azorín, M. Teresa Moral-Pumarega, Diana Escuder-Vieco et al. "Immediate pre-partum SARS-CoV-2 status and immune profiling of breastmilk: A case-control study." *Frontiers in Immunology* 12 (2021): 720716. https://doi.org/10.3389/fimmu.2021.720716

[19] Young, Bridget E., Antti E. Seppo, Nichole Diaz, Casey Rosen-Carole, Anna Nowak-Wegrzyn, Joseline M. Cruz Vasquez, Rita Ferri-Huerta et al. "Association of human milk antibody induction, persistence, and neutralizing capacity with SARS-CoV-2 infection vs mRNA vaccination." *JAMA pediatrics* 176, no. 2 (2022): 159-168. https://doi.org/10.1001/jamapediatrics.2021.4897

[20] Demers-Mathieu, Veronique, DaPra, Ciera, Mathijssen, Gabrielle, A Sela, David, M Jarvinen, Kirsi, Seppo, Antti, Fels, Shawn, Medo, Elena. 2021. "Human Milk Antibodies Against S1 and S2 Subunits from SARS-CoV-2, HCoV-OC43, and HCoV-229E in Mothers with a Confirmed COVID-19 PCR, Viral SYMPTOMS, and Unexposed Mothers." *International Journal of Molecular Sciences* 22 (4): 1749. https://doi.org/10.3390/ijms22041749

[21] Demers-Mathieu, Veronique, Anders P. Hakansson, Stephanie Hall, Sirima Lavangnananda, Shawn Fels, and Elena Medo. "Functional antibodies against SARS-CoV-2 receptor binding domain variants with mutations N501Y or E484K in human milk from COVID-19-Vaccinated,-Recovered, and-Unvaccinated women." *Breastfeeding Medicine* 17, no. 2 (2022): 163-172. https://doi.org/10.1089/bfm.2021.0232

[22] Fox, Alisa, Jessica Marino, Fatima Amanat, Kasopefoluwa Y. Oguntuyo, Jennifer Hahn-Holbrook, Benhur Lee, Susan Zolla-Pazner, and Rebecca L. Powell. "The IgA in milk induced by SARS-CoV-2 infection is comprised of mainly secretory antibody that is neutralizing and highly durable over time." *PLoS One* 17, no. 3 (2022): e0249723. https://doi.org/10.1371/journal.pone.0249723

[23] Juncker, Hannah G., Michelle Romijn, Veerle N. Loth, Eliza JM Ruhé, Sjors Bakker, Saskia Kleinendorst, Christianne JM de Groot et al. "Antibodies against SARS-CoV-2 in human milk: milk conversion rates in the Netherlands." *Journal of Human Lactation* 37, no. 3 (2021): 469-476. https://doi.org/10.1177/08903344211018185

[24] Lebrão, Cibele Wolf, Manuela Navarro Cruz, Mariliza Henrique da Silva, Letícia Veríssimo Dutra, Camila Cristiani, Fernando Luiz Affonso Fonseca, and Fabíola Isabel Suano-Souza. "Early identification of IgA anti-SARSCoV-2 in milk of mother with COVID-19 infection." *Journal of Human Lactation* 36, no. 4 (2020): 609-613. https://doi.org/10.1177/0890334420960433

[25] Liu, Wei, Yujie Liu, Zhenjun Liu, Changxin Hong, Jian Liu, Sihui Luo, and Xueying Zheng. "A case study supporting lack of SARS-CoV-2 Spread to a 3-month old infant through exclusive breastfeeding." *Journal of Human Lactation* 37, no. 2 (2021): 269-272. https://doi.org/10.1177/0890334421991072

[26] Pace, Ryan M., Janet E. Williams, Kirsi M. Järvinen, Courtney L. Meehan, Melanie A. Martin, Sylvia H. Ley, Celestina Barbosa-Leiker et al. "Milk from women diagnosed with COVID-19 does not contain SARS-CoV-2 RNA but has persistent levels of SARS-CoV-2-specific IgA antibodies." *Frontiers in immunology* 12 (2021): 801797. https://doi.org/10.3389/fimmu.2021.801797

[27] van Keulen, Britt J., Michelle Romijn, Albert Bondt, Kelly A. Dingess, Eva Kontopodi, Karlijn van der Straten, Maurits A. Den Boer et al. "Human milk from previously COVID-19-infected mothers: the effect of pasteurization on specific antibodies and neutralization capacity." *Nutrients* 13, no. 5 (2021): 1645. https://doi.org/10.3390/nu13051645

[28] Demers-Mathieu, Veronique, Ciera DaPra, and Elena Medo. "Comparison of severe acute respiratory syndrome coronavirus 2-specific antibodies' binding capacity between human milk and serum from coronavirus disease 2019-Recovered women." *Breastfeeding Medicine* 16, no. 5 (2021): 393-401. https://doi.org/10.1089/bfm.2020.0381

[29] Juncker, Hannah G., Sien J. Mulleners, Marit J. van Gils, Tom PL Bijl, Christianne JM de Groot, Dasja Pajkrt, Aniko Korosi, Johannes B. van Goudoever, and Britt J. van Keulen. "Comparison of SARS-CoV-2-specific antibodies in

human milk after mRNA-based COVID-19 vaccination and infection." *Vaccines* 9, no. 12 (2021): 1475. https://doi.org/10.3390/vaccines9121475

[30] Juncker, Hannah G., M. Romijn, Veerle N. Loth, Tom G. Caniels, Christianne JM de Groot, Dasja Pajkrt, Marit J. van Gils, Johannes B. van Goudoever, and Britt J. van Keulen. "Human milk antibodies against SARS-CoV-2: a longitudinal follow-up study." *Journal of Human Lactation* 37, no. 3 (2021): 485-491. https://doi.org/10.1177/08903344211030171

[31] Wang, Wenling, Yanli Xu, Ruqin Gao, Roujian Lu, Kai Han, Guizhen Wu, and Wenjie Tan. "Detection of SARS-CoV-2 in different types of clinical specimens." *Jama* 323, no. 18 (2020): 1843-1844. https://doi.org/10.1001/jama.2020.3786

[32] Briana, Despina D., Anna Papadopoulou, Garyfallia Syridou, Edoardo Marchisio, Eleni Kapsabeli, Anna Daskalaki, and Vassiliki Papaevangelou. "Early human milk lactoferrin during SARS-CoV-2 infection." *The Journal of Maternal-Fetal & Neonatal Medicine* 35, no. 25 (2022): 6704-6707. https://doi.org/10.1080/14767058.2021.1920010

[33] Yu, Jack C., Hesam Khodadadi, Évila Lopes Salles, Quyen Pham, Pinkal Patel, and Babak Baban. "High levels of interferon-alpha expressing macrophages in human breast milk during SARS-CoV-2 infection: A case report." *Breastfeeding Medicine* 16, no. 5 (2021): 439-442. https://doi.org/10.1089/bfm.2020.0369

[34] Pullen, Krista M., Caroline Atyeo, Ai-Ris Y. Collier, Kathryn J. Gray, Mandy B. Belfort, Douglas A. Lauffenburger, Andrea G. Edlow, and Galit Alter. "Selective functional antibody transfer into the breastmilk after SARS-CoV-2 infection." *Cell reports* 37, no. 6 (2021). https://doi.org/10.1016/j.celrep.2021.109959

[35] Huang, Yuan, Chan Yang, Xin-feng Xu, Wei Xu, and Shu-wen Liu. "Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19." *Acta Pharmacologica Sinica* 41, no. 9 (2020): 1141-1149. https://doi.org/10.1038/s41401-020-0485-4

[36] Shah, Priyanka, Gabriela A. Canziani, Erik P. Carter, and Irwin Chaiken. "The case for S2: the potential benefits of the S2 subunit of the SARS-CoV-2 spike protein as an immunogen in fighting the COVID-19 pandemic." *Frontiers in immunology* 12 (2021): 637651. https://doi.org/10.3389/fimmu.2021.637651

[37] Hall, Victoria, Sarah Foulkes, Ferdinando Insalata, Peter Kirwan, Ayoub Saei, Ana Atti, Edgar Wellington et al. "Protection against SARS-CoV-2 after Covid-19 vaccination and previous infection." *New England Journal of Medicine* 386, no. 13 (2022): 1207-1220. https://doi.org/10.1056/NEJMoa2118691

[38] Fitzsimmons, Sean P., Mishell K. Evans, Cheryl L. Pearce, Michael J. Sheridan, Raoul Wientzen, and Michael F. Cole. "Immunoglobulin A subclasses in infants' saliva and in saliva and milk from their mothers." *The Journal of pediatrics* 124, no. 4 (1994): 566-573. https://doi.org/10.1016/S0022-3476(05)83135-X

[39] Fernández-Buhigas, Irene, Nieves Rayo, Julia Cuesta Silos, Berta Serrano, Olga Ocón-Hernández, Bo Wah Leung, Juan Luis Delgado et al. "Anti-SARS-CoV-2-specific antibodies in human breast milk following SARS-CoV-2 infection during pregnancy: a prospective cohort study." *International Breastfeeding Journal* 19, no. 1 (2024): 5. https://doi.org/10.1186/s13006-023-00605-w

[40] Low, Jia Ming, Yue Wey Low, Youjia Zhong, Cheuk Yiu Charlotte Lee, Ming Chan, Nicholas Beng Hui Ng, Zubair Amin, and Yvonne Peng Mei Ng. "Titres and neutralising capacity of SARS-CoV-2-specific antibodies in human milk: a systematic review." *Archives of Disease in Childhood-Fetal and Neonatal Edition* 107, no. 2 (2022): 174-180. https://doi.org/10.1136/archdischild-2021-322156

[41] Thanigainathan, Sivam, Venkatesh Kaliyaperumal, Sindhu Sivanandan, Sasirekha Rengaraj, Rahul Dhodapkar, and Adhisivam Bethou. "Is SARS-COV-2 transmitted through breastfeeding?." *Indian journal of pediatrics* 88 (2021): 800-801. https://doi.org/10.1007/s12098-021-03681-0

[42] Leung, H. Y. H., B. W. Leung, M. M. Gil, V. Rolle, S. Moungmaithong, C. C. Wang, and L. C. Poon. "SARS-CoV-2-specific antibodies and neutralization capacity in breast milk following infection vs vaccination." Ultrasound in Obstetrics & Gynecology 60, no. 3 (2022): 425-427. https://doi.org/10.1002/uog.24965

[43] Liu, Xiyao, Haoyue Chen, Meijing An, Wangxing Yang, Yujie Wen, Zhihuan Cai, Lulu Wang, and Qianling Zhou. "Recommendations for breastfeeding during Coronavirus Disease 2019 (COVID-19) pandemic." *International Breastfeeding Journal* 17, no. 1 (2022): 28. https://doi.org/10.1186/s13006-022-00465-w

[44] Selma-Royo, Marta, Christine Bäuerl, Desirée Mena-Tudela, Laia Aguilar-Camprubí, Francisco J. Pérez-Cano, Anna Parra-Llorca, Carles Lerin, Cecilia Martínez-Costa, and Maria Carmen Collado. "Anti-SARS-CoV-2 IgA and IgG in human milk after vaccination is dependent on vaccine type and previous SARS-CoV-2 exposure: a longitudinal study." *Genome medicine* 14, no. 1 (2022): 42. https://doi.org/10.1186/s13073-022-01043-9

[45] Lackey, Kimberly A., Ryan M. Pace, Janet E. Williams, Lars Bode, Sharon M. Donovan, Kirsi M. Järvinen, Antti E. Seppo et al. "SARS-CoV-2 and human milk: What is the evidence?." Maternal & child nutrition 16, no. 4 (2020): e13032. https://doi.org/10.1111/mcn.13032

[46] Conti, Maria Giulia, Sara Terreri, Eva Piano Mortari, Christian Albano, Fabio Natale, Giovanni Boscarino, Giulia Zacco et al. "Immune response of neonates born to mothers infected with SARS-CoV-2." *JAMA Network Open* 4, no. 11 (2021): e2132563-e2132563. https://doi.org/10.1001/jamanetworkopen.2021.32563

[47] Kilic, Talat, Sebnem Kilic, Nurcan Kırıcı Berber, Ayten Gunduz, and Yasemin Ersoy. "Investigation of SARS-CoV-2 RNA in milk produced by women with COVID-19 and follow-up of their infants: A preliminary study." *International journal of clinical practice* 75, no. 7 (2021): e14175. https://doi.org/10.1111/ijcp.14175

[48] Yu, Yuanyuan, Youjiang Li, Yingying Hu, Bin Li, and Jian Xu. "Breastfed 13 month-old infant of a mother with COVID-19 pneumonia: a case report." *International breastfeeding journal* 15 (2020): 1-6. https://doi.org/10.1186/s13006-020-00305-9