Impact of Gestational COVID-19 on Neonatal Outcomes

Is Vertical Infection Possible?

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Background: The vertical transmission of severe acute respiratory coronavirus-2 (SARS-CoV-2) remains highly debated. Here, we evaluated SARS-CoV-2-transmission in newborns with intrauterine conditions.

Methods: This was a prospective, observational and multicentric study involving 13 Spanish hospitals included in the GEStational and NEOnatal-COVID cohort. Pregnant women with microbiologically confirmed SARS-CoV-2 infection during any trimester of pregnancy or delivery and their newborns were included from March to November 2020. Demographic, clinical and microbiological data were also obtained. Viral loads were analyzed in different maternal and newborn biological samples (placenta, breast milk and maternal blood; urine, meconium and newborn blood).

Results: A total of 177 newborns exposed to SARS-CoV-2 were included. Newborns were tested by reverse transcriptase-polymerase chain reaction using nasopharyngeal swabs within the first 24–48 hours of life and at 14 days of life. In total 5.1% were considered to have SARS-CoV-2 infection in the neonatal period, with 1.7% considered intrauterine and 3.4% intrapartum or early postnatal transmission cases. There were no differences in the demographic and clinical characteristics of the pregnant women and their newborns' susceptibility to infections in their perinatal history or background.

Conclusions: Intrauterine transmission of SARS-CoV-2 is possible, although rare, with early postnatal transmission occurring more frequently. Most infected newborns remained asymptomatic or had mild symptoms that evolved well during follow-up. We did not find any maternal characteristics predisposing infants to neonatal infection. All infected newborn mothers had acute infection at delivery.

Although there was no presence of SARS-CoV2 in cord blood or breast milk samples, SARS-CoV-2 viral load was detected in urine and meconium samples from infected newborns.

Key Words: gestational SARS-COV-2 infection, neonatal COVID-19, vertical transmission, placenta, breast milk

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The global spread of severe acute respiratory coronavirus (SARS-CoV-2) has confronted the scientific community with a health emergency. The anatomical, physiological and immunological changes that accompany pregnancy may increase the susceptibility of pregnant women to infections; however, the effects of SARS-CoV2 infection on pregnant women, fetuses and newborns remain uncertain.[1–3]

Three mechanisms of vertical transmission have been described: intrauterine transmission, intrapartum transmission, when infection occurs during labor, and childbirth and postnatal transmission, when transmission occurs due to respiratory or other infectious secretions through breast-feeding or through contact with other infected caregivers.[4,5] To date, it is not clear whether vertical transmission of SARS-CoV-2 is possible, as contradictory data have been published in the literature.[6,7] The rate of SARS-CoV-2 perinatal transmission is variable, with most series being around 2%–5%,[8–10] although there are publications reporting it as high as 12%,[11] with transmission being mainly postnatal.[12]

Although studies assessing vertical transmission are mostly epidemiological,[11,12] there is insufficient evidence of viral transmission in biological samples through microbiological techniques.

Our main objective was to describe the transmission of SARS-CoV-2 infection in intrauterine-exposed infants by analyzing the viral load in biological samples from mothers and newborns. The secondary objective was to describe the epidemiology and clinical features of infected pregnant women and the obstetric history and perinatal characteristics of their newborns.

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MATERIALS AND METHODS

This was a prospective, observational, descriptive and multicentric study involving 13 Spanish hospitals included in the GEStational and NEOnatal (GESNEO)-COVID cohort, which includes RECLIP (Red Española de Ensayos Clínicos Pediátricos).

Population and Study Period

Pregnant women with microbiologically confirmed SARS-CoV-2 infection during any trimester of pregnancy or delivery and their newborns were included in the study. The diagnosis of infection was made by performing reverse transcriptase-polymerase chain reaction (RT-PCR) tests on nasopharyngeal swabs. Pregnant women with positive IgG serological test results were not included in this analysis. Patients were included in the study between March 15, 2020, and November 30, 2020.

Epidemiological and Clinical Variables

Demographic and clinical information, including comorbidities and obstetric history, was collected from pregnant women. For SARS-CoV-2 infection, the time of diagnosis, clinical presentation, need for treatment, imaging tests and admission for infection were specified.

Perinatal and delivery clinical data, anthropometric data, feeding type and comorbidities during the neonatal period were collected from newborns.

Although there may be slight variations according to the protocol of each participating center, asymptomatic newborns whose mothers presented an adequate clinical condition were kept in joint isolation in the obstetrics ward. If a newborn required admission, they were was carried out in an isolation room until the result of the RT-PCR.

Newborns were classified according to the mechanism of transmission described by Blumberg et al[4] (Table 1).

Subsequently, two groups were created to compare the baseline demographic and clinical maternal characteristics and neonatal outcomes, depending on the newborns' state of infection. Group 1 was formed by noninfected newborns and their mothers, and group 2 by infected newborns (intrauterine, intrapartum or early postnatal infection) and their mothers.

Microbiologic Samples: Collection, Preservation and Processing

In pregnant women, nasopharyngeal swab for SARS-CoV-2 RT-PCR were obtained at the time of diagnosis and at delivery.

At the time of delivery, maternal blood and placental samples were collected for RT-PCR as well as umbilical cord blood samples. The samples were initially frozen and archived at the Microbiology Department of the Hospital General Universitario Gregorio Marañón until analysis. In addition, placental samples were collected in formalin for subsequent immunohistochemical analysis.

Nasopharyngeal swabs for RT-PCR were performed in newborns in the first 24–48 hours after delivery. In those with a positive result, a second test was performed immediately to confirm the results. RT-PCR of nasopharyngeal swabs was also performed on all newborns on the 14th day of life. Urine and meconium samples were collected during the first 48 hours of life.

In pregnant women who breast-fed their infants, breast milk samples were collected by hand or breast pumping after adequate breast hygiene.

Biological samples in the viral transport media were tested for the presence of SARS-CoV-2 RNA using real-time RT-PCR to detect the N gene and the *ORF1a1b* gene (TaqPath Multiplex, Thermo Fisher).

Statistical Analysis

Continuous variables are described as medians and interquartile ranges (IQR), and categorical variables as absolute frequencies and percentages. The χ^2 test or Fisher's exact test, as appropriate, was used to compare categorical variables, and the Wilcoxon rank-sum test was used for continuous variables, with $P < 0.05$, which is considered statistically significant. Data were analyzed using StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC.

Ethical Considerations

The study was approved by the Clinical Research Ethics Committee of Hospital General Universitario Gregorio Marañón (Code IRB 00006051) and all participating centers. Informed consent was obtained from the mothers or legal guardians of the newborns.

RESULTS

The evolution of 174 pregnant women with SARS-CoV-2 infection during pregnancy and 177 newborns was detailed (171 singletons, 3 twins).

The demographic and clinical characteristics of the pregnant women, as well as the obstetric-perinatal backgrounds and clinical characteristics of their newborns as compared to those of uninfected newborns are described in Tables 2 and 3.

TABLE 1. SARS-COV-2 mechanism of transmission according to Blumberg*'s* classification

Intrauterine transmission:	
One item in each of the following categories is needed.	
Nasopharyngeal RT-PCR positive in the first 24 hours of life OR RT-PCR positive in amniotic fluid OR cord blood OR neonatal blood at <24 hours.	A positive swab of the neonatal respiratory tract (nasopharynx, oropharynx or saliva) 24 hours postnatally OR positive IgM during the first week of life.
Intrapartum or early postnatal transmission:	
One item in each of the following categories is needed.	
Negative RT-PCR from a swab of the neonatal respiratory tract (nasopharynx, oropharynx or saliva) in the first 24 hours of life.	A positive swab of the neonatal respiratory tract (nasopharynx, oropharynx or saliva) 24 hours postnatally OR positive IgM during the first week of life.
Superficial exposure to SARS-CoV-2 or transient viremia:	
One item in each of the following categories is needed.	
Positive nasopharyngeal RT-PCR in the first 24 hours of life OR positive RT-PCR results in amniotic fluid OR cord blood OR neonatal blood at <24 hours.	No evidence of persistence or immune response: A positive swab of the neonatal respiratory tract (nasopharynx, oropharynx or saliva) 24 hours postnatally OR negative IgM during the first 2–3 weeks of postnatal life.
No perinatal transmission:	
Negative nasopharyngeal RT-PCR in the first 24–48 hours of life and at 2 weeks of postnatal life.	

RT-PCR, reverse transcriptase-polymerase chain reaction.

TABLE 2. Univariate analysis of the demographic and clinical characteristics of the pregnant women according to their newborn infection status

	All pregnant women n = 174	Non-infected NB's mothers n = 165 (94.8%)	Infected NB's mothers n = 9 (5.2%)	*P*
Age (years)	32.9 (28.9–36.0)	33.2 (29.2–36.5)	30.7 (26.6–33.5)	0.128
Ethnicity				0.722
Caucasian	90 (58.4%)	84 (57.5%)	7 (77.8%)	
Latino American	54 (35.1%)	52 (35.6%)	2 (22.2%)	
Arabic	8 (5.2%)	8 (5.5%)	0	
Black/Afroamerican	2 (1.3%)	2 (1.4%)	0	
GA at diagnosis (w)	37.4 (33.3–39.7)	37 (32.7–39.7)	38.7 (38.3–40.4)	0.118
Any comorbidity	49 (28.2%)	46 (27.9%)	3 (33.3%)	0.713
Symptomatology	76 (44.4%)	72 (44.4%)	4 (44.4%)	1
Respiratory symptoms severity*				0.176
Mild	16 (47.0%)	16 (48.5%)	0	
Moderate	12 (35.3%)	12 (36.4%)	0	
Severe	6 (17.7%)	5 (15.1%)	1 (100%)	
Pneumonia COVID-19	24 (13.8%)	23 (13.9%)	1 (11.1%)	1
Pneumonia at delivery	12 (6.9%)	11 (6.7%)	1 (11.1%)	0.483
Specific treatment	55 (31.6%)	52 (31.5%)	3 (33.3%)	1
COVID hospitalization	27 (15.5%)	26 (15.8%)	1 (11.1%)	1
ICU admission	4 (14.8%)	3 (11.5%)	1 (100%)	0.148
Days of admission	7 (5–12)	7 (5–12)	30	0.148
RT-PCR + at delivery	129 (74.1%)	120 (72.7%)	9 (100%)	0.286

*Severity of respiratory infection was stratified into mild (upper respiratory tract symptoms), moderate (pneumonia confirmed by chest X-ray without signs of severity) and severe (presence of hypoxemia with partial oxygen saturation [$SatO_2$] <90%, acute confusional state, or arterial hypotension).

GA indicates gestational age; ICU, intensive care unit; NB: newborn.

Continuous variables are described as the median and interquartile range (IQR). Categorical variables as absolute frequencies and percentages. p<0.05 is considered statistically significant.

Microbiologic Data

At the time of delivery, 39% of pregnant women had an acute infection (RT-PCR positive, IgG negative), 30% had a recent infection (RT-PCR positive, IgG positive) and 31% had a past infection (RT-PCR negative, IgG positive with RT-PCR positive during pregnancy).

A total of 115 maternal blood samples and 81 placenta samples were collected for RT-PCR, which revealed only 1 case of viral load in the blood sample and placenta from 1 pregnant woman. These samples belonged to a 33-year-old pregnant woman of Latin American origin with acute infection at the time of delivery, whose mild clinical symptoms (fever, headache and catarrhal symptoms) started 48 hour before delivery. The newborn remained asymptomatic, and all collected samples (nasopharyngeal swab, cord blood, urine, meconium and breast milk) were negative for SARS-CoV-2.

The immunohistochemical analyses of all placental samples for SARS-CoV-2 (16) were negative.

Seventy-nine breast milk samples were tested, and no viral loads were detected in any of the samples.

All RT-PCR results for cord blood and newborn blood samples (64) were negative. The viral load was detected in 3 newborn urine samples and 3 meconium samples. All cases were newborns with acute SARS-CoV-2 infection diagnosed by positive RT-PCR of nasopharyngeal swabs in their first 48 hours of age.

Characteristics of Newborns with COVID-19 and Mechanism of Transmission

All newborns (177) were tested using RT-PCR of nasopharyngeal swabs in the first 24–48 hours after delivery and at 14 days of life. A total of 159 neonates had negative RT-PCR results; therefore, they were considered to be noninfected newborns. Twelve infants with positive RT-PCR results on nasopharyngeal swabs were identified. The microbiological and clinical data from neonates and their mothers are summarized in Table 4.

According to the Blumberg classification, the infection of newborns 1–3 was classified as resulting from the intrauterine transmission, 4–9 from intrapartum or early postnatal transmission and 10–12 from nasopharyngeal secretal contamination or transient viremia. After excluding cases of contamination by nasopharyngeal secretions or transient viremia, 5.1% (9) of newborns were diagnosed with SARS-CoV-2 infection in the neonatal period, 1.7% (3) had contracted it intrauterinely and 3.4% (6) contracted it in the intrapartum or early postnatal.

All were born to mothers who were acutely infected at the time of delivery. None of the pregnant women had any viral load in the maternal blood or placenta samples.

Only 2 out of 9 infected newborns presented symptoms, both suffering respiratory distress that evolved satisfactorily at follow-up.

Maternal and Neonatal Characteristics Compared According to Newborn Infection Rates

There were no differences in the demographic and clinical characteristics of the pregnant women compared to the newborns (Table 2). No differences were found in the perinatal history or background of the newborns (Table 3). Compared with noninfected newborns, infected newborns did not develop more symptoms and did not have higher admission rates to the neonatal intensive care unit. Infected newborns were more frequently fed a combination of formula and breastmilk compared to noninfected newborns, with no differences in the rates of artificial formula feeding.

DISCUSSION

In our large cohort of infants born to mothers with SARS-CoV-2 infection during pregnancy, 5.1% had neonatal infections, with infections being more prevalent in the early postnatal period. We did not find viral loads in any of the samples of cord blood, placenta or breast milk collected from infected newborns.

TABLE 4. Microbiological and clinical data of newborns with COVID-19 by the mechanism of transmission

	Microbiological stools			Symptomatology	
NB	Nasopharyngeal swab RT-PCR	Maternal samples	NB samples	Mothers	NBs
Intrauterine transmission (RT-PCR positive <24 h and later)					
1	Positive (days +0, +2, +12)		Cord blood – Urine – Meconium +	No	Respiratory distress (24 hours need oxygen)
2	Positive (days +0, +2, +17)	Plasma –	Urine + Meconium –	Headache	No
3	Positive (days +0, +3, +7)		Urine + Meconium **+**	No	Respiratory distress and central apneas *29 week preterm
Intrapartum or early postnatal transmission (RT-PCR negative < 48 h and positive later)					
4	Negative (day +1), positive (day +16, +30)			Fever, dyspnea	No
5	Negative (day +1), positive (day +16)	Plasma – BM –	Urine – Meconium –	No	No
6	Negative (day +1), positive (day +14)			Fever	No
7	Negative (day +1), positive (day +15)	BM –	Urine – Meconium –	No	No
8	Negative (day +0), positive (day +13)			No	No
9	Positive (days +2, +16, +24)	Plasma – BM –	Urine + Meconium **+**	Fever, asthenia	No
Nasopharyngeal secretions contamination or transient viremia (RT-PCR positive < 24 h but negative later)					
10	Positive (day +1), negative (day +3).			Headache	No
11	Positive (day +0), negative (day +1, +15).	Plasma – Placenta –	Cord blood – Meconium –	No	No
12	Positive (day +1), negative (day +7)	Plasma – Placenta – BM –	Cord blood – Urine – Meconium –	No	Isolated febrile peak

BM, breast milk; NB, newborn; RT-PCR, reverse transcriptase-polymerase chain reaction; w, weeks.

The vertical transmission of SARS-CoV-2 remains highly debated at the present time, with some studies showing controversial results, and most published studies are case reports or retrospective research.[13] In our cohort of newborns exposed to SARS-CoV-2 during pregnancy, 5.1% had neonatal infections, a similar result to those in other previously published studies.[8–10] In a systematic review and meta-analysis by Di Toro et al,[14] 11 neonates out of 275 tested positive for SARS-CoV-2 (5%). Comparable results were described by Kotylar et al[15] in their systematic review and meta-analysis, with an infection rate of 3.2% and 48 positive neonates out of 936 neonates. Moreover, they differentiated between studies from China and Europe, with higher rates of vertical transmission in reported European studies than in Chinese studies (4.9% in the United Kingdom vs. 2% in China).[16]

TABLE 3. Univariate analysis of the obstetric-perinatal history and clinical characteristics of the newborns according to the newborn's infection status

	All NB n = 177	Noninfected NBs n = 168 (94.9%)	Infected NBs n = 9 (5.1%)	*P*
Sex (female)	93 (52.5%)	88 (52.4%)	5 (55.5%)	1
GA (w)	39.3 (38.1–40.3)	39.4 (38.1–40.3)	38.7 (38.4–40.4)	0.918
Prematurity rate	29 (16.4%)	27 (16.1%)	2 (22.2%)	0.643
Late preterm	14 (48.3%)	13	1	
32–33 weeks	6 (20.7%)	6	0	
29–31 weeks	5 (17.2%)	4	1	
<28 weeks	4 (13.8%)	4	0	
Type of delivery				0.815
Eutocic	115 (65.0%)	109 (64.9%)	6 (66.7%)	
Instrumental	19 (10.7%)	19 (11.3%)	0	
Cesarean section	43 (24.3%)	40 (23.8%)	3 (33.3%)	
Symptomatology	40 (22.6%)	37 (22.0%)	3 (33.3%)	0.424
Need for NICU admission	16 (9.0%)	15 (8.9%)	1 (11.1%)	0.583
Breast-feeding method				0.006
Maternal	116 (65.6%)	114 (67.9%)	2 (22.2%)	
Artificial	19 (10.7%)	18 (10.7%)	1 (11.1%)	
Mixed	42 (23.7%)	36 (21.4%)	6 (66.7%)	

GAincidates gestational age; NB, newborn; NICU, neonatal intensive care unit.

Continuous variables are described as medians and interquartile ranges (IQR). Categorical variables are presented as absolute frequencies and percentages. Statistical significance was set at $P < 0.05$.

Classifying newborns according to the mechanism of transmission is difficult[4] due to the great heterogeneity in the definitions of vertical transmission. One of the first classifications was proposed by Blumberg et al,[4] which was used in our study. Subsequently, new classifications were published, including by the World Health Organization (WHO),[5] which were updated in February 2021.

There is growing interest in establishing whether the intrauterine transmission is possible. In our study and according to Blumberg's classification, positivity in RT-PCR nasopharyngeal swabs in the first 24 hours of life and its subsequent persistence allows a newborn's infection to be classified as occurring via intrauterine transmission. This differs from the WHO's classification, where a positive sterile sample in the first 24–48 hours of life (cord blood, placenta, amniotic fluid, bronchoalveolar lavage or cerebrospinal fluid) for "confirmed" transmission is required, with only "possible" transmission identified from nasopharyngeal exudate samples. In our series, we did not obtain placental or amniotic fluid samples from any of the newborns classified by intrauterine transmission, and only 1 of the cases was RT-PCR performed on cord blood, which was negative. According to the WHO's classification, our 3 newborns should be categorized as having "possible" intrauterine transmission.

The second potential transmission mechanism is intrapartum or early postnatal transmission. Blumberg et al grouped these two mechanisms together, whereas the WHO classification differentiates between intrapartum and early postnatal transmission according to the timing of microbiological testing: a positive test for intrapartum transmission occurs between 24 and 48 hours of life, requiring a negative test in the first 24 hours, and early postnatal transmission is defined when this occurs 48 hours after birth. The narrow timeline separating the 2 mechanisms makes it exceedingly difficult to differentiate between them. In both cases, the diagnosis can be established using nasopharyngeal samples, without the strict necessity of positive sterile samples.

In our study, 6 newborns with intrapartum or early postnatal transmission were included. Five had a negative diagnostic test in the first 48 h of life, with a positive RT-PCR at the follow-up visit at 2 weeks of life, corresponding to early postnatal transmission per the WHO classification. The sixth case involved a newborn whose mother was RT-PCR positive in the first 24 hours postpartum. The newborn's RT-PCR results were positive at 48 hours of life. According to the WHO classification, this case would be classified as a "possible" early postnatal transmission in the absence of a previous negative diagnostic test.

The last transmission mechanism proposed by Blumberg et al., contamination by nasopharyngeal secretions or transient viremia, was attributed to 3 newborns in our sample. The WHO classification classifies these cases as undetermined within the intrauterine transmission. However, in most cases, the first nasopharyngeal swab of the newborn was performed after skin-to-skin contact with the mother and joint isolation in the same room. The lack of viral persistence implies that we cannot consider these newborns as infected, which is why they were not included in the subsequent analysis.

Table 5 summarizes the differences between the mechanism of transmission classifications proposed by Blumberg and the WHO in our sample of newborns with positive RT-PCR results.

The clinical and epidemiological characteristics of the pregnant women in our series were similar to those reported in other studies,[9,17] and there were no differences when compared according to neonatal infection status. All mothers of infected newborns had an acute infection at the time of delivery, suggesting that transmission occurs mainly by the end of the gestation period and during delivery and that there are no maternal risk factors that contribute to transmission to newborns. In our sample, neither the severity of maternal infection nor the presence of symptoms was related to neonatal infection. In any case, due to the small number of infected newborns, these conclusions should be taken with caution. To the best of our knowledge, no prospective studies have described the presence of certain maternal characteristics that predispose neonates to infection.

Table 5. Comparison of the mechanisms of newborn SARS-COV-2 transmission using the Blumberg[4] and WHO[5] classifications

	Blumberg *et al's* classification	WHO classification
1	Intrauterine	"Possible" intrauterine
2	Intrauterine	"Possible" intrauterine
3	Intrauterine	"Possible" intrauterine
4	Intrapartum or early postnatal	Confirmed early postnatal
5	Intrapartum or early postnatal	Confirmed early postnatal
6	Intrapartum or early postnatal	Confirmed early postnatal
7	Intrapartum or early postnatal	Confirmed early postnatal
8	Intrapartum or early postnatal	Confirmed early postnatal
9	Intrapartum or early postnatal	"Possible" early postnatal
10	Contamination by nasopharyngeal secretions or transient viremia	Indeterminate
11	Contamination by nasopharyngeal secretions or transient viremia	Indeterminate
12	Contamination by nasopharyngeal secretions or transient viremia	Indeterminate

In our cohort, we found no differences in gestational age, type of delivery, symptomatology and need for admission between infected and noninfected newborns. The most common neonatal symptoms of COVID-19 described in the literature are tachypnea, milk regurgitation, cough, vomiting and fever.[18] In our study, of the 9 infected newborns, only 2 had symptoms (22.2%): transient tachypnea and respiratory distress syndrome of prematurity. Owing to the high frequency of these symptoms in neonatal units, we cannot be sure that this clinical presentation was due to SARS-CoV-2 infection. None of the infected newborns had fever or digestive symptoms.

A systematic review published by Mirbeik et al.,[8] including 17 articles with microbiological data found no evidence of SARS-CoV-2 in the placenta, cord blood or breast milk samples. On the other hand, a recent systematic review and meta-analysis published in 2021 found 1 cord blood sample and 2 placental samples positive for SARS-CoV-2. In our cohort, we only found viral loads of SARS-CoV-2 in 1 blood sample and 1 placental sample, both from a pregnant woman with acute infection at the time of delivery. As these were samples from a pregnant woman with mild symptoms, and because vertical transmission to the newborn was not subsequently observed, it is possible that contamination occurred in the analysis of the samples.

In a study by Elbow et al.,[19] in which 62 maternal blood and newborn cord blood samples and 44 placentas from infected mothers were analyzed, no evidence of SARS-CoV-2 RNA was found. We did not detect SARS-CoV-2 in any of the placental samples. Several studies have analyzed the anatomopathological alterations in the placentas of pregnant women with COVID-19, without finding significant differences concerning uninfected pregnant women.[20,21] A study by Levitan et al[20] found no evidence of the virus when immunohistochemically analyzing placental samples.

At the beginning of the pandemic, there was considerable controversy about whether breast-feeding should be allowed because it was unknown whether it could be a possible route of transmission of the virus. In accordance with the recommendations of the Spanish Society of Neonatology,[22] the WHO,[23] and the American Academy of Pediatrics,[24] in our cohort of Spanish hospitals,

breast-feeding was maintained in infected mothers while following the relevant hygienic measures, including the use of maternal masks while in contact with the newborn and by employing hand and breast hygiene.

We found no viral loads in the breast milk samples. To date, several studies have assessed the presence of the virus in breast milk, with controversial results.[25,26] Moreover, these studies had a small sample size. Grob et al.,[27] were one of the first to find viral RNA in serial samples of milk from an infected mother. Some studies with larger numbers of patients, such as the 1 by Pace et al,[28] in which samples from 18 women were studied, found no viral RNA in serial milk samples from infected mothers. In our cohort, infected newborns were less frequently exclusively breast-fed, with the possible loss of the long-term benefits of breast milk, although these results should be interpreted with caution owing to the small sample size.

There are published studies in adult patients[29] and pediatric populations[30] where the virus was isolated in urine and fecal samples, and viral loads in fecal excretions can even be maintained for weeks after infection. In our sample, the virus was detected by RT-PCR in the urine and meconium of 4/9 (44.4%) newborns with PCR-confirmed infection in the nasopharyngeal exudate.

One of the main limitations of this study is the heterogeneity in the recruitment of patients; at the beginning of the pandemic, only pregnant women with symptoms were tested by RT-PCR, leading to a loss of asymptomatic pregnant women that could potentially have been included in the first month of the study. In addition, we did not have blood, placental or milk samples from all dyads included in the study. In addition, our study only included live newborns; therefore, we have no information on whether infection during pregnancy could cause miscarriage or intrauterine fetal death.

The strengths of this study include its large sample size and multicentric nature. Another notable feature is that our study allowed us to understand the natural history of SARS-CoV-2 infection without the influence of vaccination status, as it was conducted at the beginning of the pandemic.

CONCLUSIONS

In our large prospective study, the intrauterine transmission of SARS-CoV-2 was possible, although rare, with early postnatal transmission through direct contact with infected persons occurring more frequently. Most infected newborns remain asymptomatic or have mild symptoms with good subsequent evolution during follow-up. We did not find any maternal epidemiological characteristics that predisposed one to neonatal infection, although we observed that infected newborns were from mothers with acute infection at delivery.

The presence of viremia in maternal blood and placenta samples was anecdotal in our cohort, and the virus was not found in the cord blood or newborn blood samples. The possibility of viral transmission through breast milk is unlikely, as no viral load was detected in the samples studied; therefore, breast-feeding is not contraindicated in cases of infection. Finally, the virus can be detected in the urine and meconium samples of infected newborns.

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