Infants Born Following SARS-CoV-2 Infection in Pregnancy

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abstract

OBJECTIVES: To evaluate outcomes of neonates born to mothers with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during pregnancy, the dynamics of placental transfer of maternal antibodies, and its persistence during infancy.

METHODS: Cohort study enrolling neonates born to mothers with SARS-CoV-2 infection in pregnancy. All infants were evaluated at birth. Those born to women with infection onset within 2 weeks before delivery were excluded from further analyses. Remaining infants underwent cerebral and abdominal ultrasound, fundoscopy evaluation, and were enrolled in a 12 month follow-up. Qualitative immunoglobulin G (IgG)/immunoglobulin M and quantitative IgG to S1/S2 subunits of spike protein were assessed in mother–neonate dyads within 48 hours postdelivery and during follow-up.

RESULTS: Between April 2020 and April 2021, 130 of 2745 (4.7%) neonates were born to mothers with SARS-CoV-2 infection in pregnancy, with 106 of 130 infections diagnosed before 2 weeks before delivery. Rates of preterm and cesarean delivery were comparable between women with and without infection (6% vs 8%, $P = .57$; 22% vs 32%, $P = .06$). No clinical or instrumental abnormalities were detected at birth or during follow-up. There was a positive correlation between maternal and neonatal SARS-CoV-2 IgG levels ($r = 0.81$, $P < .001$). Transplacental transfer ratio was higher after second-trimester maternal infections as compared with first and third trimester ($P = .03$). SARS-CoV-2 IgG level progressively decreased in all infants, with 89 of 92 (97%) infants seronegative at 6 months of age.

CONCLUSIONS: Clinical outcomes were favorable in all infants. Matching peak IgG level after infection and higher IgG transplacental transfer might result in the most durable neonatal passive immunity.

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WHAT'S KNOWN ON THE SUBJECT: In utero transmission of SARS-CoV-2 is considered rare but possible. SARS-CoV-2 infection in pregnancy has been associated with an increased risk of preterm birth, cesarean deliveries, and other adverse pregnancy outcomes. Transplacental transfer of SARS-CoV-2 maternal antibodies has been documented.

WHAT THIS STUDY ADDS: Long-term outcomes were favorable in all SARS-CoV-2–exposed infants, including those born to mothers with severe disease and first-trimester infections. Transplacental transfer ratio was higher after second-trimester infections. Most infants lost maternal antibodies by 6 months of age.

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Respiratory viruses are usually not easily transmitted in utero, and no evidence for in utero transmission of other coronaviruses was previously described.[1–3] Recent literature, however, poses a challenge to that assumption because in utero mother-to-child transmission of severe respiratory syndrome coronavirus 2 (SARS-CoV-2) seems to be possible even if rare.[4] The mechanisms of in utero transmission are unclear, but might be related to the presence of a viraemia in some individuals, to placental compromise with leakage of maternal blood into amniotic fluid and the expression of the cell membrane-associated, angiotensin-converting enzyme 2 receptor, and transmembrane protease serine-2 in placental cells, although there are no definitive data on their coexpression and expression by gestational age (GA).[5–8] SARS-CoV-2 infection during pregnancy has been associated with placental vascular damage, which might facilitate the transplacental passage of the virus and affects fetal perfusion,[9–11] even though placental involvement does not necessarily translate into fetal infection or damage.

Previous studies reported that maternal antibodies produced in response to SARS-CoV-2 infection cross the placenta, likely conferring some degree of passive protection to neonates.[12–17] The efficiency of placental transfer of maternal antibodies in relation to GA at infection and the duration of this possible protection are still unclear.

The aim of this study is to evaluate the short- and long-term outcomes of neonates after SARS-CoV-2 infection during pregnancy, the efficiency and dynamics of placental transfer of maternal antibodies to the neonate, and its persistence during the first months of life.

MATERIALS AND METHODS

Study Population

Neonates born to mothers with SARS-CoV-2 infection during pregnancy, delivered at IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, between April 2020 and April 2021, were included.

During the study period, all women were routinely screened for SARS-CoV-2 infection on admission for delivery. Between April 2020 and October 2020, given to the recent spread of the virus, SARS-CoV-2 infection during pregnancy was defined by the presence of SARS-CoV-2 antibodies in maternal serum at delivery with or without previous history of SARS-CoV-2 exposure or symptoms. Between November 2020 and April 2021, clinical data consistent with coronavirus disease 2019 (COVID-19) during pregnancy and/or history of SARS-CoV-2 exposure confirmed by a positive nasopharyngeal swab for SARS-CoV-2 RNA were required to confirm infection during pregnancy.

Preterm neonates and neonates born to mothers with peripartum SARS-CoV-2 infection, defined as a first positive nasopharyngeal swab for SARS-CoV-2 RNA between 2 weeks before delivery and 2 days postdelivery, and without SARS-CoV-2 antibodies, were excluded from the follow-up and serological study, but data on the mode and timing of delivery were included in the analysis. The study was approved by the hospital institutional review board.

Definitions and Data Collection

For mothers, data regarding demographics, trimester of infection, illness severity, and comorbidities were collected at delivery.

Timing of maternal infection was based on the first SARS-CoV-2–positive test during pregnancy, supported by clinical, microbiological, and anamnestic data.

Illness severity was defined per definition provided by the US Centers for Disease Control and Prevention:

1. asymptomatic;
2. mild disease: symptoms other than shortness of breath, dyspnea, or radiographic evidence of pneumonia;
3. moderate disease: evidence of lower respiratory disease and saturation of oxygen (SpO2) $\geq$94% on room air;
4. severe disease: evidence of lower respiratory diseases with pulse oxygen saturation $<$94% on room air, or lung infiltrated $>$50%; and
5. critical disease.[18]

Results of SARS-CoV-2 RNA testing in maternal serum, cord blood, and placental tissues at delivery were collected, when available.

For neonates, data regarding GA, birth weight (BW), length, head circumference, clinical signs at birth, and results of the hearing screening test performed by otoacoustic emissions were collected. All neonates underwent nasopharyngeal swab for SARS-CoV-2 RNA within 24 hours of life, and blood tests for total and differential blood cell count, hemoglobin and platelets evaluation, and liver function tests within 48 hours of life.

Cerebral ultrasound, fundoscopy evaluation, and abdominal ultrasound were performed during the first 3 months of life.

All term infants were enrolled in a follow-up program with evaluation at 1, 3 (±30 days), 6, 9 (±30 days) and 12 months of age. The follow-up

did not include a formal neurodevelopmental assessment.

SARS-CoV-2 antibodies were assessed in both maternal and neonatal serum within 48 hours postdelivery. SARS-CoV-2 antibodies were repeatedly assessed during follow-up until negative. Also, at the same time points, the mothers were evaluated for SARS-CoV-2 antibodies.

According to the definition provided by the World Health Organization (WHO), in utero SARS-CoV-2 transmission requires evidence of maternal SARS-CoV-2 infection any time during pregnancy, in utero fetal SARS-CoV-2 exposure, and SARS-CoV-2 persistence or immune response in the neonate. Timing of vertical transmission is classified as confirmed, possible, unlikely, indeterminate.[2]

Laboratory Methods

Blood samples were collected in ethylenediaminetetraacetic acid-anticoagulated tubes. The tests' procedures and interpretation were those reported in the manufacturer instructions for the assays.

For the qualitative detection of SARS-CoV-2, immunoglobulin G (IgG) and immunoglobulin M (IgM), the SARS-CoV-2 IgM and IgG CLIA kits (Shenzen YHLO Biotech Co, Ltd., China) were used. The assays were performed on the iFlash3000 CLIA analyzer. The assay detects antibodies to nucleocapsid and spike proteins.[19] For the quantitative detection of SARS-CoV-2 antibodies, the LIAISON SARS-CoV-2 S1/S2 IgG CLIA assay (DiaSorin S.p.A., Saluggia, Italy) performed on the LIAISON XL Analyzer (DiaSorin) was used. The cutoff value for a positive result is 15 arbitrary unit (AU)/mL. The assay detects antibodies to the S1/S2 subunits of the spike protein.[19] The qualitative assay was used in all dyads within the first 48 hours postdelivery; the quantitative assay was used at all time points to monitor the antibody amount over the months.

For SARS-CoV-2 RNA detection, 3 commercially available kits were used: Xpert Xpress SARS-CoV-2 assay (Cepheid, USA), Simplexa COVID-19 Direct kit (DiaSorin, Italy), and Allplex SARS-CoV-2 Assay (Seegene, Korea). All tests were performed according to the manufacturer's recommendations.

Statistical Analysis

Demographics, and clinical and serological data were summarized using descriptive analyses. Categorical data were reported using numbers and percentages. Continuous variables were reported using mean and SD, or median, range, and/or interquartile range (IQR), as appropriate.

Transplacental transfer ratio was calculated as infant IgG concentration divided by maternal IgG concentration at birth. Correlations between maternal and neonatal IgG concentrations and between transplacental transfer ratio and persistence of IgG in the first months of life were reported using the Pearson correlation coefficient (r). Standard descriptive analyses, including Fisher's exact test, unpaired t test, Mann-Whitney U test, and Wilcoxon signed rank test, were used as appropriate to compare demographics, clinical characteristics, transplacental transfer ratio, and maternally derived IgG persistence among groups on the basis of disease severity and timing of infection.

Statistical significance was set at $P < .05$. Stata version 15 (StataCorp, College Station, TX, USA) was used for analyses.

RESULTS

During the study period, 2745 neonates were born, of whom 130 of 2745 (4.7%) were born to 130 mothers with SARS-CoV-2 infection during pregnancy. Twenty-four dyads were peripartum SARS-CoV-2 infections (Fig 1).

Most neonates were born via vaginal delivery, whereas 28 of 130 (22%) were born by cesarean delivery. All cesarean deliveries were performed because of pregnancy-related indications. In the study period, the rate of cesarean delivery among women with and without SARS-CoV-2 infection was similar (22% vs 32%, $P = .06$). The number of preterm births was similar among women with and without SARS-CoV-2 infection (6% vs 8%, $P = .57$).

Overall, 106 of 2745 (4%) neonates were born to 106 mothers with SARS-CoV-2 infection diagnosed >2 weeks before delivery.

Sixteen of 106 (15%) infants were born between April 2020 and October 2020; 90 of 106 (85%) were born between November 2020 and April 2021.

Mothers

Maternal demographic characteristics are summarized in Table 1.

Most women (76 of 106, 72%) were symptomatic during pregnancy, mainly with mild diseases (67 of 106, 63%, Table 1). Of the 30 of 106 (28%) asymptomatic women, 14 were identified because of serological screening during the first phase of the study and were unaware of their serological status for SARS-CoV-2 at delivery. The other asymptomatic women were tested during pregnancy because of history of SARS-CoV-2 exposure. None of the enrolled women was SARS-CoV-2–vaccinated.

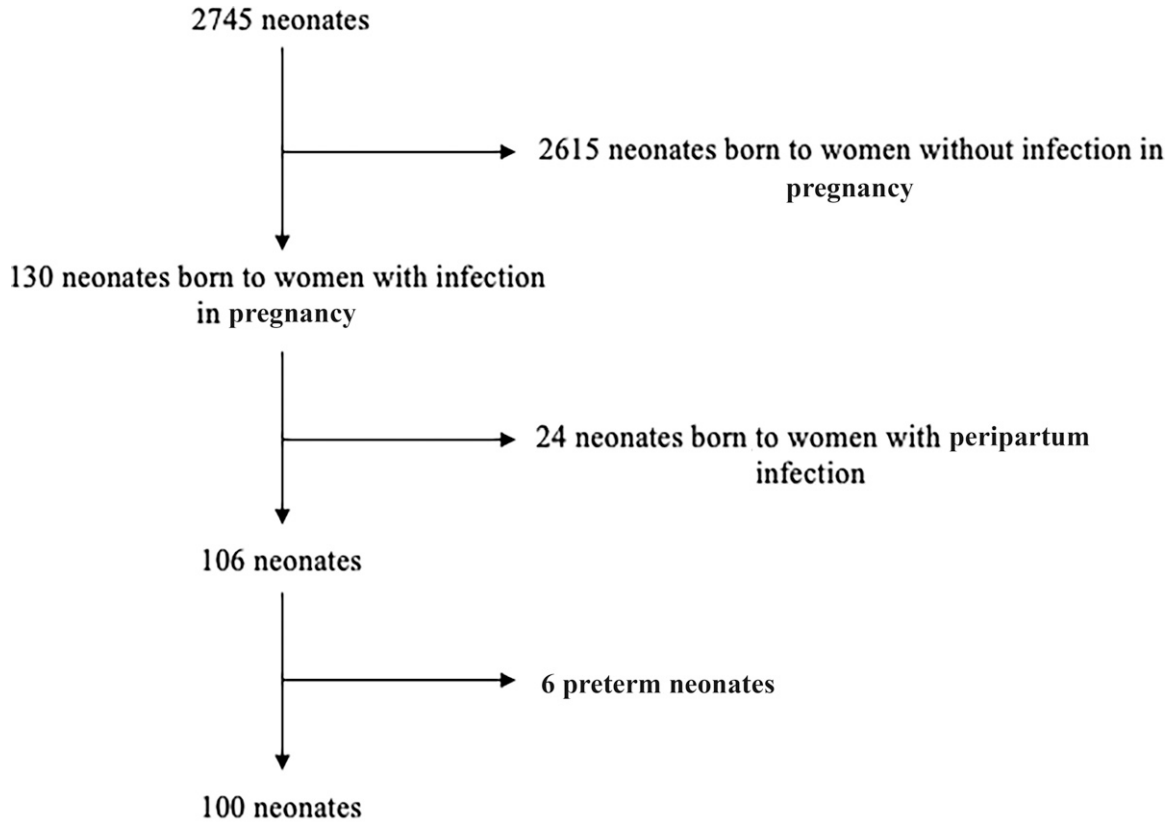

FIGURE 1
Flow diagram of the selection process of the study population.

The trimester of infection was known in 92 of 106 (87%) women (Table 1).

Thirty-two placental biopsies were analyzed for SARS-CoV-2 RNA. Only 1 of 32 (3%) tested positive, from a woman with a mild disease, with disease onset 8 weeks before delivery.

Neonates

All 106 neonates had a negative nasopharyngeal swab for SARS-CoV-2 RNA within 24 hours of life.

Median GA at birth was 39 weeks (range 25^{+5}–41^{+4}). Mean BW was 3305 g (SD 468), mean length 49.8 cm (SD 2.5), and mean head circumference 33.9 cm (SD 1.6). Six of 106 (6%) neonates were born preterm (Table 2) and were excluded from the follow-up and serological study.

No congenital abnormalities SARS-CoV-2–related were detected.

Laboratory parameters at birth, including hemoglobin, platelets, total and differential white blood cell count, and liver function tests, were normal for age in all but 1 infants, found to have a low hemoglobin value (hemoglobin 9.8 g/dL). This neonate was born to a mother with a third-trimester infection and required a blood cell transfusion; the anemia gradually resolved over the first months of life. All the most common causes of neonatal anemia were excluded.

All infants underwent cerebral ultrasound and no abnormalities SARS-CoV-2–related were detected. Fundoscopy evaluation and abdominal ultrasound were performed in 99 of 100 (99%) and 95 of 100 (95%) infants (Fig 2), and no pathologic findings were detected. All infants passed the hearing screening.

All infants reached the 12-month follow-up. During follow-up, growth parameters were in the normal range in all infants. None of the infants suffered of recurrent respiratory infections, nor of SARS-CoV-2 infections.

Serology

Matched maternal–neonatal blood sera collected within 48 hours postdelivery were available for all 100 dyads.

All mothers had detectable SARS-CoV-2 IgG; 13 of 100 mothers (13%) were both IgG- and IgM-positive at delivery (trimester of infection: first 1 of 8, 12%; second 1 of 8, 12%; third 6 of 8, 75%; not known in 5 women).

TABLE 1 Maternal Characteristics by Clinical Presentation of SARS-CoV-2 Infection During Pregnancy

Characteristics	Asymptomatic ($N = 30$)	Symptomatic			Total ($N = 106$)
		Mild ($N = 67$)	Moderate ($N = 4$)	Severe ($N = 5$)	
Age at delivery, y					
Mean (SD)	30.9 (6.3)	33.6 (5.4)	31.7 (4.9)	32.8 (2.3)	32.7 (5.7)
No. (%)					
18–24	7 (23)	4 (6)	0	0	11 (10)
25–29	5 (17)	8 (12)	1 (25)	0	14 (13)
30–34	8 (27)	23 (34)	2 (50)	3 (60)	36 (34)
35–39	9 (30)	26 (39)	1 (25)	2 (40)	38 (36)
≥40	1 (3)	6 (9)	0	0	7 (7)
Birth country, No. (%)					
Europe	16 (53)	57 (85)	3 (75)	5 (100)	81 (76)
Asia	10 (33)	6 (9)	0	0	16 (15)
Africa	1 (3)	3 (4)	1 (25)	0	5 (5)
South America	3 (10)	1 (2)	0	0	4 (4)
Other	0	0	0	0	0
Gravidity, median (IQR)	1 (1–2)	2 (1–2)	2	1 (1–2)	2 (1–2)
Prepregnancy comorbidities, No. (%)	9 (30)[a]	11 (16)[a]	0	1 (20)[a]	21 (20)[a]
Trimester of infection, No. (%)					
First	4 (13)	6 (9)	1 (25)	1 (20)	12 (11)
Second	3 (10)	28 (42)	2 (50)	0	33 (31)
Third	9 (30)	33 (49)	1 (25)	4 (80)	47 (44)
Not known	14 (47)	0	0	0	14 (13)
Gestational diabetes, No. (%)	9 (30)	11 (16)	0	2 (40)	22 (21)
IgG-positive, IgM-negative at delivery, No. (%)	22 (73)	63 (94)	3 (75)	4 (80)	92 (87)
IgG-positive, IgM-positive at delivery, No. (%)	8 (27)	4 (6)	1 (25)	1 (20)	14 (13)

[a] Some women may have >1 prepregnancy comorbidity. The most common comorbidity was hypothyroidism (11 women, 10%), followed by venous thrombosis (3%), thrombocytopenia (2%), factor II mutation (2%), factor V Leiden (1%), polycythemia (1%), lupus (1%), obesity (1%), mastocytosis (1%), and asthma (1%).

Maternal serum was analyzed for SARS-CoV-2 RNA in 10 of 100 women, and 1 of 10 sample (10%) from an asymptomatic women with infection onset 5 weeks before delivery tested weakly positive.

Maternal SARS-CoV-2 S1/S2 IgG level at delivery was significantly higher in women with third-trimester infections as compared with women with first- and second-trimester infections (median 46 AU/mL, IQR 23–77, vs 25 AU/mL, IQR 15–49, $P = .046$, Fig 3), but was not significantly different between asymptomatic and symptomatic mothers (median 35.5 AU/mL, IQR 18–55, vs 36 AU/mL, IQR 22–77, $P = .23$).

Ninety-six neonates had detectable SARS-CoV-2 IgG at birth (Table 2). Considering only term infants, 92 of 100 (92%) had detectable IgG. One neonate had both detectable SARS-CoV-2 IgG and IgM, further confirmed at a second sample taken within the first week of life. This neonate was born to a mother with a third-trimester infection and both SARS-CoV-2 IgG and IgM positivity at delivery. Neonatal nasopharyngeal swab, meconium, stools, blood, and urine were negative for SARS-CoV-2 RNA. This infant fulfilled the WHO criteria for possible in utero infection.

TABLE 2 Neonatal Characteristics by Maternal Clinical Presentation of SARS-CoV-2 Infection During Pregnancy

Characteristics	Asymptomatic Mothers ($N = 30$)	Symptomatic Mothers ($N = 76$)	P
GA, median (wk:d range)	39 (32:0–41)	39 (25:5–41:4)	.39
Preterm delivery (GA <37 wk), No. (%)	1 (3)	5 (6.5)	.67
BW, mean (SD)	3309 (447)	3304 (477.2)	.96
Small for GA, No. (%)	1 (3)	5 (6.5)	.67
Mode of delivery, No. (%)			
Vaginal	21 (70)	61 (80)	.30
Cesarean	9 (30)	15 (20)	.30
Apgar score at 5 min ≥9, No. (%)	30 (100)	75 (99)	.99
IgG-positive, IgM-negative at birth, No. (%)	28 (93)	67 (88)	.72
IgG-positive, IgM-positive at birth, No. (%)	0	1 (1)	.99
IgG-negative, IgM-negative at birth, No. (%)	2 (7)	8 (11)	.72
IgG level at birth, AU/mL,[a] median (IQR)	18.5 (12–49.2)	27 (13–73)	.13
Transfer ratio, mean (SD)[a]	0.73 (0.38)	0.93 (0.59)	.09

[a] Quantitative IgG level and transfer ratio are reported only for the 100 term infants.

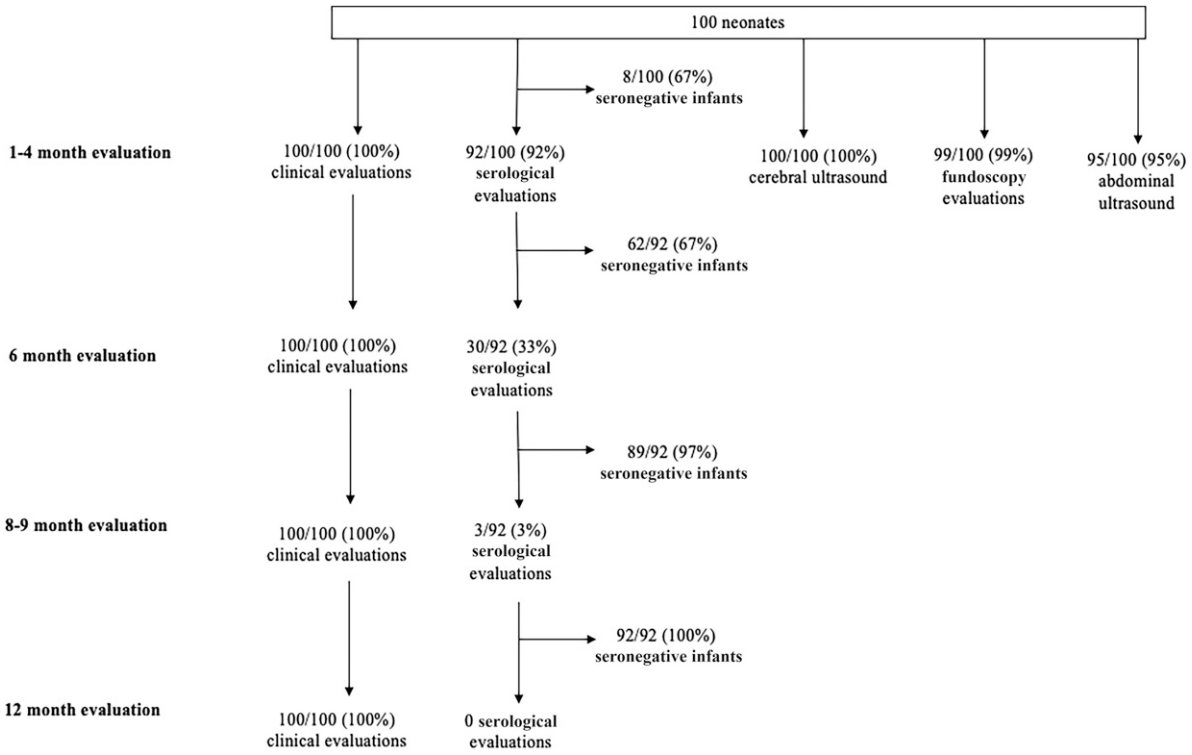

FIGURE 2
Diagram showing the number of clinical, serological, and instrumental evaluations of the 100 enrolled infants performed at each time point.

There was a positive correlation between maternal and neonatal SARS-CoV-2 IgG levels at first sampling ($r = .81$, $P < .001$).

Neonatal SARS-CoV-2 S1/S2 IgG level at birth was not significantly different in relation to the trimester of maternal infection (median 25.5 AU/mL, IQR 17.5–68, vs 19.5 AU/mL, IQR 15–60, vs 31 AU/mL, IQR 15–70.5, $P = .09$, Fig 3), nor between neonates born to asymptomatic and symptomatic mothers (Table 2).

Transplacental transfer ratio was significantly higher when maternal infection had occurred in the second trimester compared with first- and third-trimester infections ($P = .03$, Table 3), but was similar between asymptomatic and symptomatic women (Tables 2, 3).

SARS-CoV-2 S1/S2 IgG level progressively decreased during follow-up in all infants (Figs 4, 5). Considering the 92 term infants with detectable SARS-CoV-2 S1/S2 IgG at birth, 49 of 92 (53%) had lost maternal antibodies at 3 months of age, and this percentage increased to 67% (62 of 92) and 97% (89 of 92) at 4 and 6 months of age, respectively (Fig 2). All infants had lost maternal antibodies by 8 months of life. The neonate with possible in utero infection lost antibodies at 6 months of age.

The persistence of maternal antibodies was positively correlated to SARS-CoV-2 S1/S2 IgG level at first sampling ($r = .66$; $P < .001$).

The SARS-CoV-2 S1/S2 IgG level was followed at 1 to 3 months and 6 months postdelivery in 81 of 100 (81%) and 17 of 100 (17%) women, respectively. The trimester of infection was known in 69 of 81 women (first trimester 7 of 69, second trimester 24 of 69, third trimester 38 of 69) and 13 of 17 women (first trimester 1 of 13, second trimester 4 of 13, third trimester 8 of 13). The median IgG level at 1 to 3 months was similar to IgG level at delivery (median 36 AU/mL, IQR 20–65, vs 44 AU/mL, IQR 22–99, $P = .18$), whereas it was significantly higher at 6 months (124 AU/mL, IQR 43.5–306.5, $P = .001$ and $P = .01$, Fig 4), even if the median time between SARS-CoV-2 infection and sampling was 255.5 days (range 126–371).

DISCUSSION

Current evidence suggests that in utero transmission of SARS-CoV-2 infection is a possible but rare event. As per definition provided by the WHO, specimens from both maternal and fetal tissues are necessary to confirm in utero

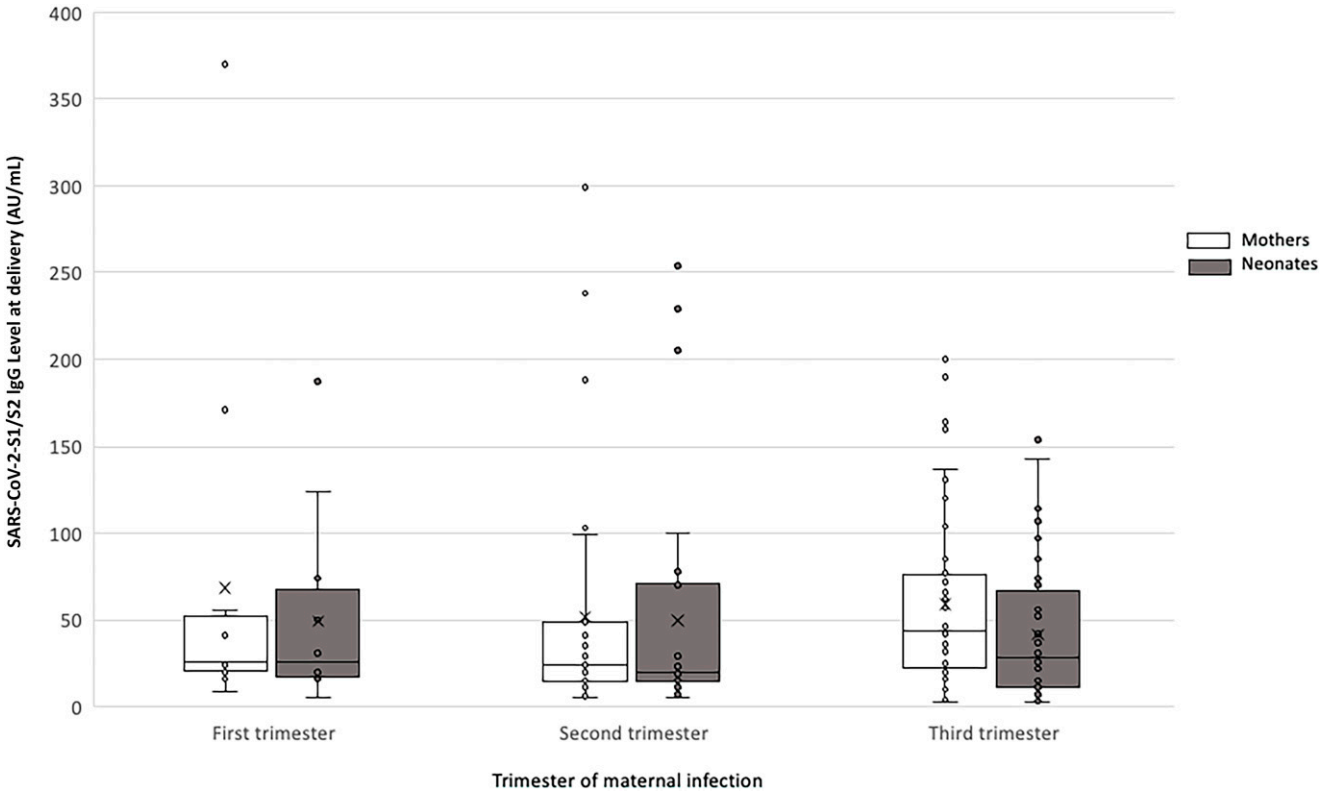

FIGURE 3
Distribution of maternal and neonatal SARS-CoV-2 S1/S2 IgG at the time of delivery in relation to trimester of infection. Note: The box represents the middle 50% of the data; the line within the box represents the median; whiskers indicate variability outside the upper and lower quartile, excluding outliers.

infection. These samples were not routinely collected in this study, limiting the possibility to assess the potential for in utero transmission of SARS-CoV-2 in this cohort. Only 1 neonate was defined as possible in utero infection,[2] although the low sensitivity and specificity of IgM to diagnose congenital infections suggest caution in the interpretation of this finding because it could reflect an aberrant transplacental IgM transfer related to placental compromise. Similarly, other authors reported on the possible presence of IgM in neonatal cord blood and negative birth specimens for SARS-CoV-2 RNA, underlining the complexity of providing a clear categorization of in utero infections.[12,20]

All the enrolled exposed neonates had no clinical abnormalities detected at birth or during follow-up, including those born to mothers with severe COVID-19 and with first-trimester infections. Most women were infected during the second wave of the pandemic in Italy, similarly to the rate of infections in the general population,[21] and most of them had asymptomatic or mild infections. During the study period, both the rate of preterm birth and cesarean deliveries were comparable between neonates born to mothers with and without SARS-CoV-2 infection. Previous studies led to conflicting results: although some authors suggested a significant increase of adverse pregnancy outcomes, including preterm birth, low

TABLE 3 Transplacental Transfer Ratio in Relation to Type and Timing of Maternal Infection for the 100 Term Infants

	Type of Infection		Trimester of Infection			
Characteristics	Asymptomatic (N = 29)	Symptomatic (N = 71)	First (N = 11)	Second (N = 30)	Third (N = 45)	Not Known (N = 14)
Transplacental TR, No. (%)						
<0.5	9 (30)	22 (29)	3 (25)	4 (12)	17 (36)	4 (29)
0.5–1	14 (47)	34 (45)	4 (33)	16 (48)	22 (47)	6 (43)
1.01–2.00	7 (23)	18 (24)	5 (42)	11 (33)	8 (17)	4 (29)
>2.00	0	2 (33)	0	2 (6)	0	0
Mean transplacental TR (SD)	0.73 (0.38)	0.93 (0.59)	0.98 (0.46)	1.06 (0.59)	0.74 (0–48)	0.77 (0.36)
Median transplacental TR (IQR)	0.73 (0.44–1.03)	0.75 (0.51–1.2)	0.95 (0.61–1.24)	0.98 (0.69–1.23)	0.64 (0.44–0.99)	0.80 (0.45–1.13)

TR, transfer ratio.

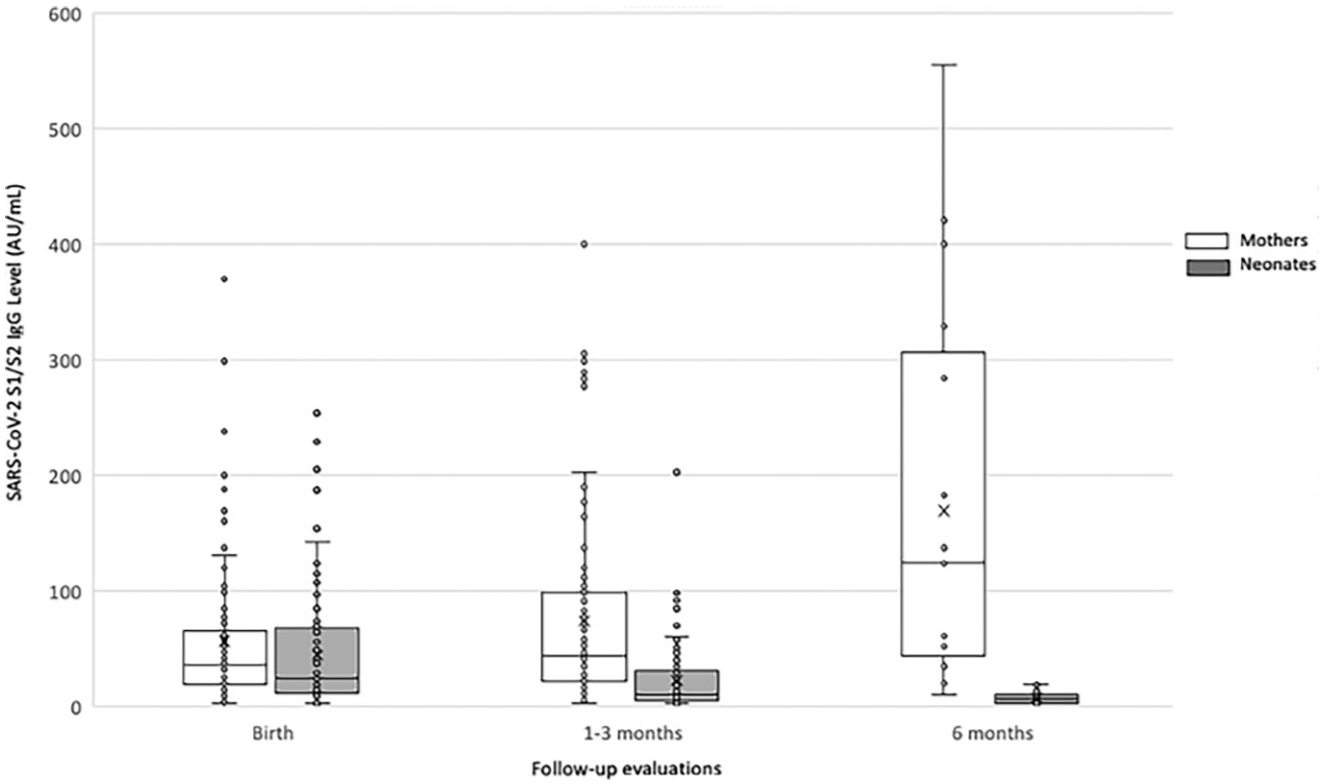

FIGURE 4

Distribution of maternal and neonatal SARS-CoV-2 S1/S2 IgG during follow-up, showing a progressive decrease in neonatal blood and a progressive increase in a subset of maternal samples. Note: The box represents the middle 50% of the data; the line within the box represents the median; whiskers indicate variability outside the upper and lower quartile, excluding outliers.

BW, and cesarean deliveries among SARS-CoV-2–infected women,[22–24] others did not,[25] in line with these data. The rate of possible SARS-CoV-2–related miscarriages or stillbirths in the study period was not collected.

Neonatal outcomes after in utero SARS-CoV-2 exposure have been mainly described in neonates born to mothers infected during late pregnancy. One strength of this study is that the trimester of infection was known in most women and that neonates born to mothers with infections in all 3 trimesters of gestations were included, providing reassuring evidence of the lack of signs directly or indirectly related to maternal infection. Indeed, even though intrauterine transmission is recognized as a rare event, previous studies suggested that the placenta might be vulnerable to SARS-CoV-2 and the possibility of perinatal morbidity without infection related to placental immune activation and damage.[9,11,26] In light of the possible unfavorable outcome related to both these events, all infants underwent cerebral and abdominal ultrasound, and no abnormalities were detected. Unfortunately, placentas were not systematically analyzed. Also, even though multiple efforts were conducted to correctly assess timing of maternal infection, it is possible that some infections were incorrectly allocated. The long-term outcomes, up to 12 months of age, was favorable. It will be interesting to confirm this observation on larger sample size and for a longer follow-up period.

Hematologic parameters were in the normal range for age in all but 1 neonate, who was born anemic. Autoimmune hemolytic anemia has been rarely described in patients with COVID-19, mostly in moderate to severe disease.[27,28] The underlying mechanism has yet to be elucidated, but it might be related to the immunologic and inflammatory activation secondary to viral infection. Some authors also suggested a possible role of molecular mimicry, with the structural similarities between the erythrocyte membrane protein ankyrin 1 and the viral S protein being key factors of immunologic cross-reactivity.[29] Since this neonate had maternally derived antibodies, the hypothesis of a link between maternal infection and neonatal anemia may not be completely rejected.

Transplacental acquired antibodies are considered a useful arm of the neonatal immune defense. The extent to which maternal SARS-

Timing	No. samples	No. seronegative samples	Median IgG level (IQR)
≤48 hours of life	100	8	35 (19-73)
1 month	92	17	29 (16.2-54.7)
3 month (± 30 days)	75	45	22 (16.-41.5)
6 month	30	27	15 (12-19.2)
9 month (± 30 days)	3	3	-

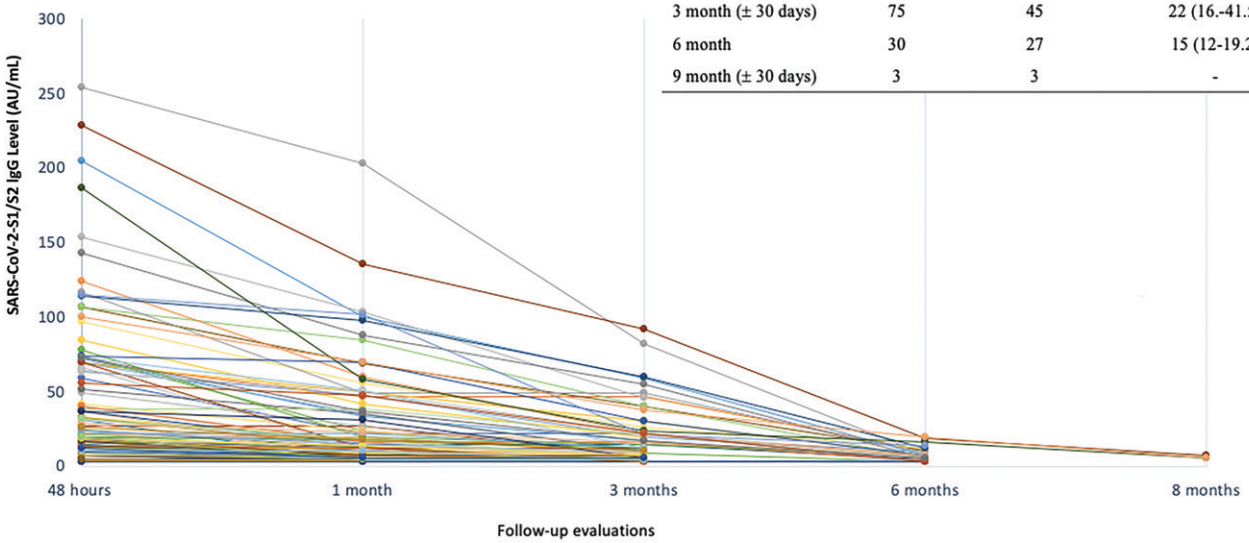

FIGURE 5
Graph and table showing the maternal IgG decay in the 100 term infants.

CoV-2 antibodies cross the placenta is important for understanding a potential mechanism of neonatal protection from COVID-19 and, ideally, for developing appropriate maternal vaccination strategies aimed at protecting both mothers and neonates. In this study, even if maternal IgG level at delivery was higher after third-trimester SARS-CoV-2 infections, higher transplacental IgG transfer ratios were observed when maternal SARS-CoV-2 infection occurred in the second trimester. This result is in line with previous reports, suggesting an impaired transplacental transfer when maternal infection occurs during late gestation[13,16,17]; however, it might also reflect a limited time available for IgG passage. Matching the higher IgG transplacental transfer ratio and the peak response after infection might result in the highest and most durable neonatal passive immunity. Whether transplacental passage of vaccine-induced antibodies overlaps with that seen after natural infection remains to be established.

The different transplacental transfer ratio likely drives the lack of correlation between the trimester of maternal infection and the IgG amount in neonates at delivery. Indeed, the IgG amount of neonates born to mothers with infection during the first, second, and third trimester of pregnancy showed an important overlap (Fig 1). Thus, even though transplacental transfer ratio is higher when maternal infection occurs during the second trimester, maternal antibodies are detectable in neonates also when infection occurs in other trimesters, including late gestation up to 14 days before delivery.

Previous studies showed that IgG production starts 12 to 14 days after SARS-CoV-2 infection, peaks 3 to 7 weeks after infection, and then plateaus and persists for at least 8 weeks,[30] even if peak timing might be longer in pregnancy, around 60 to 120 days.[15] In the current study, even if postdelivery IgG levels were followed only in few women, a delayed IgG peak was observed, with a progressive increase of the IgG level until 56 weeks postinfection. This delayed response may be related to multiple factors, such as the peripheral immune adaptation during pregnancy needed to balance fetal tolerance and growth with host defense. However, because of the small sample available during follow-up, this observation should be further studied and possibly verified on a larger sample.

The level of neonatal maternally derived IgG at birth correlated with maternal IgG level, and this level correlated with the duration of passive immunity in infants. The persistence of these antibodies showed a wide variability, but most infants lost maternal antibodies at 3 months of age and all infants lost antibodies within 8 months of age. No previous studies on large samples are available on

the dynamics of maternally derived SARS-CoV-2 IgG, but it is possible that there may be some variability based on the sensitivity and specificity of the serological assay.[19] Even though it remains to be elucidated whether the presence of maternally derived SARS-CoV-2 IgG correlates with neonatal protection, it is known that vertically transferred immunity can interfere with infant humoral response to vaccination, although the T-cell response is usually unaffected.[31] Maternal antibodies against SARS-CoV-2 were rapidly eliminated after birth. These results may contribute to understand the vulnerability of infants to SARS-CoV-2 and could assist the planification of appropriate vaccination strategies in infants, as soon as studies on the safety and efficacy of SARS-CoV-2 vaccines in this population become available.

ABBREVIATIONS

AU: arbitrary unit
BW: birth weight
COVID-19: coronavirus disease 2019
GA: gestational age
IgG: immunoglobulin G
IgM: immunoglobulin M
IQR: interquartile range
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
WHO: World Health Organization

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