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Vertical transmission of SARS-CoV2 during pregnancy: A high-risk cohort

Mariane de Fátima Yukie Maeda[1] | Maria de Lourdes Brizot[1] | Maria Augusta Bento Cicaroni Gibelli[2] | Silvia Maria Ibidi[2,3] | Werther Brunow de Carvalho[2,3] | Mara Sandra Hoshida[1] | Clarisse Martins Machado[4] | Ester Cerdeira Sabino[5] | Lea Campos de Oliveira da Silva[6] | Thomas Jaenisch[7,8] | Maria Cássia Jacintho Mendes-Correa[4] | Philippe Mayaud[9] | Rossana Pulcinelli Vieira Francisco[1,3] | HC-FMUSP-Obstetric COVID19 Study Group

[1]Disciplina de Obstetrícia, Departamento de Obstetrícia e Ginecologia da FMUSP, São Paulo, Brazil

[2]Disciplina de Neonatologia, Departamento de Pediatria da FMUSP, São Paulo, Brazil

[3]Hospital Universitário da Universidade de São Paulo, São Paulo, Brazil

[4]Laboratório de Virologia – LIM 52, Instituto de Medicina Tropical da FMUSP, São Paulo, Brazil

[5]Departamento de Moléstias Infecciosas e Parasitárias, Instituto de Medicina Tropical da FMUSP, São Paulo, Brazil

[6]Laboratório de Medicina Laboratorial – LIM 03, Hospital das Clínicas da FMUSP, São Paulo, Brazil

[7]Center for Global Health, Colorado School of Public Health, Aurora, Colorado, USA

[8]Heidelberg Institute for Global Health (HIGH), Heidelberg University Hospital, Heidelberg, Germany

[9]Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Correspondence
Rossana Pulcinelli Vieira Francisco, Departamento de Obstetricia e Ginecologia - Hospital das Clinicas, Instituto Central, 10o andar, sala 10093, Av. Dr Enéas de Carvalho Aguiar, 255 - São Paulo, SP CEP 05403 000, Brazil.
Email: rossana.francisco@hc.fm.usp.br

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Abstract

Objective: Identify the potential for and risk factors of SARS-CoV-2 vertical transmission.

Methods: Symptomatic pregnant women with COVID-19 diagnosis in whom PCR for SARS-CoV-2 was performed at delivery using maternal serum and at least one of the biological samples: cord blood (CB), amniotic fluid (AF), colostrum and/or oropharyngeal swab (OPS) of the neonate. The association of parameters with maternal, AF and/or CB positivity and the influence of SARS-CoV-2 positivity in AF and/or CB on neonatal outcomes were investigated.

Results: Overall 73.4% (80/109) were admitted in hospital due to COVID-19, 22.9% needed intensive care and there were four maternal deaths. Positive RT-PCR for SARS-CoV-2 was observed in 14.7% of maternal blood, 13.9% of AF, 6.7% of CB, 2.1% of colostrum and 3.7% of OPS samples. The interval between COVID-19

HC-FMUSP-Obstetric COVID-19 Study Group: Adriana Lippi Waissman; Aline Scalisse Bassi; Amanda Wictky Fabri; Ana Claudia Rodrigues Lopes Amaral de Souza; Ana Claudia Silva Farche; Ana Maria Kondo Igai; Ana Maria da Silva Sousa Oliveira; Carlos Eduardo do Nascimento Martins; Cristiane de Freitas Paganoti; Danielle Rodrigues Domingues; Fernanda Cristina Ferreira Mikami; Fernanda Spadotto Baptista; Jacqueline Kobayashi Cippiciani; Jéssica Gorrão Lopes Albertini; Joelma Queiroz de Andrade; Juliana Ikeda Niigaki, Marco Aurélio Knippel Galletta; Mariana Yumi Miyadahira, Mariana Vieira Barbosa; Monica Fairbanks de Barros; Nilton Hideto Takiuti; Sckarlet Ernandes Biancolin Garavazzo; Silvio Martinelli; Tiago Pedromonico Arrym; Ursula Trovato Gomez; Veridiana Freire Franco.

symptoms and delivery was inversely associated with SARS-CoV-2 positivity in the maternal blood ($p = 0.002$) and in the AF and/or CB ($p = 0.049$). Maternal viremia was associated with positivity for SARS-CoV-2 in AF and/or CB ($p = 0.001$). SARS-CoV-2 positivity in the compartments was not associated with neonatal outcomes. **Conclusion:** Vertical transmission is possible in pregnant women with COVID-19 and a shorter interval between maternal symptoms and delivery is an influencing factor.

Key points

What is already known about this topic?

- Few studies systematically evaluated the presence of SARS-CoV-2 in biological samples such as amniotic fluid, cord blood, placenta and colostrum
- Vertical transmission is still uncertain in pregnant women with COVID-19

What this study add?

- Vertical transmission is possible in pregnant women with COVID-19
- Maternal viremia is associated with positivity in amniotic fluid and/or cord blood
- A shorter interval between symptoms and delivery is an influential factor for vertical transmission

1 | INTRODUCTION

Although the burden of coronavirus disease 2019 (COVID-19) for individuals with particular conditions, such as chronic diseases, has been described to some extent,[1] the impact of disease and/or the virus on pregnant women and their offspring is not very well documented. In addition, it is not well known whether the disease at an acute stage or severe disease around the time of delivery have different impacts on pregnancy outcomes and vertical transmission.[2–10]

The co-expression of ACE-2 receptor and TMPRSS2 protease, required for SARS-CoV-2 cellular entry, is not presented at the maternal-fetal interface. However, it is possible that the damage caused by the virus in the placenta, like vascular malperfusion and inflammation, may contribute to disruption of the placental barrier, predisposing to transplacental infection.[11] Therefore, it is reasonable that some conditions related to COVID-19 infection or to the pregnancy could predispose to these changes in the placenta and facilitate the vertical transmission.

Several reports of vertical transmission have been published, mostly based on case reports, case series, or meta-analysis and reviews of such series.[2–4,12,13] Conversely, few studies have systematically investigated the presence of SARS-COV-2 in biological samples such as cord blood (CB), placental tissue, and amniotic fluid (AF) to support the role of vertical transmission.[4,6,14–18] Moreover, the results of these reports have been inconsistent, which may be due to the timing of sample collection (during the acute or post-recovery phase of maternal COVID-19), type of assays used to detect the presence of the virus, lack of test accuracy,[19,20] and the absence of a unique and standardized classification of vertical transmission.[21,22]

Given the lack of data on the potential of, timing, circumstances and risk factors influencing vertical transmission of SARS-CoV-2, we conducted a prospective cohort study of pregnant women with COVID-19 at different stages of pregnancy using systematic collection of samples (maternal blood, CB, AF and colostrum) obtained at delivery reflecting the exposition of the fetus and the neonate to maternal infection. Similar to infection with other viruses, a deeper understanding of the mechanisms and timing of SARS-CoV-2 transmission during pregnancy will allow the development of better prevention and management strategies among pregnant women.

2 | METHODS

This analysis of vertical transmission is one of the aims of a major cohort study, "exploratory study on COVID-19 in pregnant women," which began on April 12th at Hospital das Clinicas (HC) and Hospital Universitario (HU) of São Paulo University and is ongoing. The exploratory study also included estimating the seroprevalence of SARS-CoV-2 at delivery at HU.

When the COVID-19 pandemic started in São Paulo, our institution organized the HC as a COVID-19 hospital and the HU as a non-COVID-19 hospital. Therefore, pregnant women with COVID-19 or flu symptoms or contact with a SARS-CoV-2-positive person would be seen at HC; others would be seen at HU. In addition, at HU, a triage system at the hospital entrance was established; pregnant women with COVID-19 or flu symptoms or contact with a SARS-CoV-2-positive person were referred immediately to HC at any time during pregnancy, delivery or puerperium. Patients seen at HC

were only allowed to follow the antenatal or puerperium at HU after 14 days of quarantine and without symptoms.

For the present investigation, pregnant women who fulfilled the following inclusion criteria were selected: (1) singleton pregnancies with live fetuses with a diagnosis of COVID-19 during pregnancy or at delivery by real time polymerase chain reaction (RT-PCR) using a nasopharyngeal swab (NPS) or serology and with symptoms of COVID-19; and (2) investigation of SARS-CoV-2 by RT-PCR using maternal blood and at least one biological sample at delivery (CB, AF) or after birth (colostrum or oropharyngeal swab [OPS] from the neonate).

All symptomatic pregnant women admitted to HC were investigated for SARS-CoV-2 infection by RT-PCR using samples collected from the respiratory tract (nasopharynx and/or trachea) from the third day of symptoms. In cases with negative results, NPS testing was repeated, or SARS-CoV-2 serology was performed after the eighth day of symptoms. A positive RT-PCR or serology was assigned as SARS-CoV-2 infection, considering that all patients had symptoms and that PCR lacks sensitivity in late infections.[20]

The following symptoms were considered COVID-19 symptoms: fever or chills, cough, dyspnea, fatigue, myalgia, sore throat, headache, congestion, or runny nose; loss of taste or smell; diarrhea.

At HC, pregnant women with COVID-19 symptoms were evaluated by our clinical staff and hospitalized if they presented any of the following situations: (1) need for clinical support or (2) other obstetric emergencies such as hypertensive disorders, labor, premature rupture of membranes or fetal distress. Severe COVID-19 cases were defined as those who needed supplemental oxygen (because of dyspnea, respiratory frequency ≥24 breaths per minute; and/or oxygen saturation level <95%) or required admission to the intensive care unit. Delivery was indicated based on obstetrics and/or maternal clinical worsening in cases of severe COVID-19. The mode of delivery was chosen according to obstetrics and maternal clinical conditions. To avoid contamination of the neonate, delayed cord clamping and skin-to-skin contact were not allowed in cases of delivery at HC. In addition, neonates were separated from their mothers and other family members, and breastfeeding was not allowed until hospital discharge.

Information on patient demographics and history as well as details of treatments and results from exams were recorded in a REDCap platform database.

The following samples were obtained at delivery from pregnant women with symptoms of COVID-19 during pregnancy or at delivery: maternal blood, CB and AF. Maternal blood samples were collected through venipuncture immediately before delivery. Cord artery or vein blood samples (5–10 ml) were collected by needle puncture after cord clamping. The serum and plasma were separated and aliquoted. AF was collected before the rupture of amniotic membranes via direct needle aspiration using a 20-ml syringe, during cesarean section or vaginal delivery. Within 48 h of delivery, a research staff member collected a 5-ml colostrum sample into a sterile tube after appropriate cleaning of the nipples and breasts. All samples were stored at −80°C until analysis.

For neonates born at HC, a swab was collected from the oropharynx and trachea (if the newborn was intubated), or two samples from the oropharynx were collected at 48 h after delivery for SARS-CoV-2 RT-PCR analysis. In cases of any positive test for the neonate, an additional sample was obtained at 72 h after delivery and tested to exclude false-positive results. Neonates born at HU were not subjected to SARS-CoV-2 investigation, as the mothers were not symptomatic at delivery, and breastfeeding was allowed.

2.1 | Laboratory methods

Nucleic acid was extracted from 140 µl of clinical samples using a QIAmp Viral RNA mini kit (Qiagen), eluted in 60 µl and stored at −80°C until processing. Detection of viral RNA was performed using a qualitative RT-PCR—RealStar SARS-CoV-2 RT-PCR kit 1.0 RUO (Altona Diagnostic) according to the manufacturer's instructions. The reaction targets the β-coronavirus E gene and SARS-CoV-2 S gene. The assay was performed using a LightCycler 96 Instrument (Roche).

A serological test (IgG/IgM antibodies) was performed using the Wondfo One Step COVID-19 test (Guangzhou Wondfo Biotech). The test was performed using 10 µl of serum pipetted into the sample cavity of the test device, after which 80 µl was added to the cavity below the sample cavity. The result was read in 15 min by three people that had received appropriate training. The color change was compared to the assay standard.[23]

For viral culture, Vero cells (ATCC CCL-81) were used as previously described.[24–26] Vero CCL81 cells were cultured in Dulbecco minimal essential medium supplemented with heat-inactivated fetal bovine serum (10%) and antibiotics/antimycotics (Cultilab).

For virus isolation, samples were inoculated in Vero cell culture in plastic bottles (jet biofilm, 12.5 cm^2 area, 25 ml capacity) immediately after processing. The inoculated cultures were grown in a 37°C incubator in an atmosphere of 5% CO_2. The cell cultures were maintained for at least 2 weeks and observed daily for evidence of the cytopathic effect. At least two subcultures were performed weekly. Presumptive detection of virus in supernatants showing cytopathic effect was investigated using an inverted microscope (Nikon) and confirmed by specific RT-PCR as described above.

2.2 | Statistical analysis

Quantitative continuous variables are presented as means and standard deviations or medians and minimum and maximum values. Categorical variables are presented as absolute frequencies and percentages. Comparison between groups was performed using Fisher's exact test for unpaired samples with normal distribution; when the distribution was not normal, the Mann–Whitney U nonparametric test was employed. Differences were considered significant when the *p*-value was less than 0.05. The data were analyzed

using Statistical Package for the Social Sciences (SPSS version 20 IBM).

3 | RESULTS

Between 12th April and 30th September, 1044 pregnant women were admitted at HC and HU, and 595 pregnant women being assessed; 109 fulfilled the inclusion criteria for the present analysis (Figure 1). Details regarding the demographics, obstetrics and clinical characteristics of the participants are presented in Table 1. For 108 cases, the median interval between COVID-19 symptoms and delivery was 23.5 days (7.2–76.2). In one case, the patient experienced COVID-19 symptoms during pregnancy but was not able to provide the timing; therefore, this case was not included in this analysis. The majority of this cohort were admitted to hospital due to COVID-19 (80/109; 73.4%), 34.9% (38/109) required oxygen supply, 22.9% (25/109) needed intensive care and there were four maternal deaths (3.7%).

Rates of RT-PCR positivity for SARS-CoV-2 in biological samples collected at delivery were 14.7% (16/109) for maternal blood, 13.9% (6/43) for AF and 6.7% (7/105) for CB. Overall, there were some difficulties regarding the collection of AF due to the severity of some cases and the need to ascertain that the samples were not contaminated with maternal fluids during delivery, which contributed to the small number of AF samples collected. In total, 2.1% (2/97) of the maternal colostrum samples were positive for SARS-CoV-2 by RT-PCR. Viral culture was performed in these two positive cases and did not show viral replication, which may be due to the high fat composition of colostrum, which can render this analysis difficult.

Distributions of SARS-CoV-2 status in the investigated compartments are presented in Table S1. Details of the cases with at least one positive sample for SARS-CoV-2 are described in Table 2.

All pregnant women with positive SARS-CoV-2 RT-PCR results for maternal blood at delivery presented COVID-19 symptoms in the third trimester ($p = 0.007$; Table 3). In addition, a significantly shorter interval between the occurrence of COVID-19 symptoms and delivery (7.5 vs. 29 days, $p = 0.002$) was observed in cases of SARS-CoV-2 positivity in maternal blood, and this result was also observed when considering intervals ≤10 or >10 days ($p = 0.006$; Table 3). Conversely, no influence of other maternal, COVID-19-related, or obstetric factors was observed (Table 3).

Table 4 provides the associations of maternal COVID-19 and obstetrics parameters with SARS-CoV-2 RT-PCR positivity in AF and/or CB at delivery. The interval between the onset of COVID-19 symptoms and delivery was associated with positive SARS-CoV-2 RT-PCR results in AF and/or CB at delivery (7 vs. 27 days, $p = 0.049$), which was also observed for intervals ≤10 or >10 days ($p = 0.032$; Table 4). In addition, SARS-CoV-2 RT-PCR positivity in maternal serum at delivery was associated with SARS-CoV-2 RT-PCR

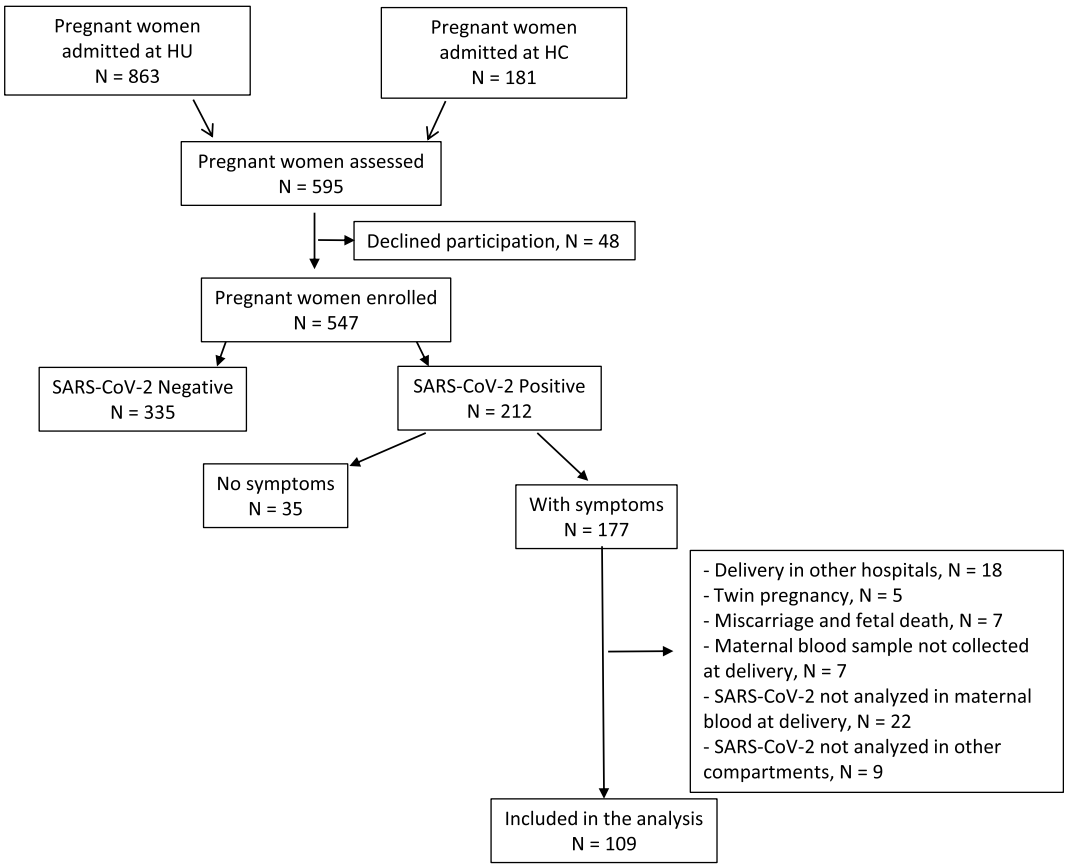

FIGURE 1 Flow-chart of study population

TABLE 1 Study population demographic, clinical, obstetrical, and neonatal characteristics

Characteristics	*N* = 109 *n* (%), mean (SD), median (range)
Maternal age, years	29.5 (7.3)
Body mass index (*n* = 107)	30.5 (18.7–48.3)
Smoking habit	10 (9.2)
Prepregnancy morbidity	
Hypertension	14 (12.8)
Diabetes	4 (3.7)
Other[a]	31 (28.4)
Obstetrics history	
Nulliparous	35 (32.1)
Preeclampsia	10 (9.2)
Gestational diabetes	21 (19.3)
Maternal COVID-19/SARS-CoV-2 parameters	
Diagnosis by RT-PCR NPS	79 (72.5)
Diagnosis by serology	30 (27.5)
Gestational age at COVID-19 symptoms, weeks (*N* = 108)	31.2 (5–40.6)
Interval between symptoms and delivery, days (*N* = 108)	23.5 (2–242)
Hospital admission due to COVID-19	80 (73.4)
Length of hospital stay, days (*N* = 80)	7 (1–187)
Required oxygen supply	38 (34.9)
ICU due to COVID-19	25 (22.9)
Severe COVID-19[b]	40 (36.7)
Maternal deaths	4 (3.7)
Delivery parameters	
Gestational age at delivery, weeks	37.9 (27.1–41.1)
Caesarean section	79 (72.5)
Length of hospital stay after delivery, days	3 (1–86)
Neonate parameters	
Birth weight, grams	3040 (680–3040)
Apgar score at 5 min <7	10 (9.2)
ICU admission	34 (31.2)
Required mechanical ventilation	10 (9.2)
Length of hospital stay, days	4 (2-190)
Breastfeeding during hospital stay	50 (45.9)
Neonatal death	0

Abbreviations: ICU, intensive care unit; NPS, nasopharyngeal swab; RT-PCR, real time polymerase chain reaction; SD, standard deviation.

[a]Other: cardiac disease, lung disease, hypothyroidism, anemia, neurological disorders.

[b]Severe COVID-19: required oxygen supply or ICU admission.

positivity in AF and/or CB (54.5% vs. 10.2%; $p = 0.001$). There was no influence observed for other maternal, COVID-19-related, or obstetric factors.

To investigate whether a positive SARS-CoV-2 RT-PCR result in AF and/or CB influences neonatal outcome, we analyzed the association between neonatal parameters and positive and negative

TABLE 2 Details of cases with at least one positive biological sample for SARS-CoV-2 at delivery ($N = 109$)

Case	Sample	COVID-19	GA_S	GA_D	Maternal blood	Cord blood	Amniotic fluid	Colostrum	Neonate Swab
1	Swab	Severe	32.86	33.57	+	+	+	–	+
2	Swab	Mild	38.14	38.57	–	+	–	+	+
3	Swab	Mild	38.43	39.43	–	+	NA	–	–
4	Swab	Mild	29	38.43	–	+	+	–	NA
5	Swab	Mild	30.14	33.71	+	+	NA	–	–
6	Swab	Severe	25.57	39.14	–	+	NA	–	NA
7	Swab	Severe	38.28	39.14	+	+	NA	–	–
8	Swab	Severe	34	35.14	+	–	+	–	–
9	Swab	Severe	30.14	39.86	–	–	+	–	NA
10	Swab	Mild	37.71	38	+	–	+	NA	–
11	Swab	Mild	39.28	40	+	NA	+	–	–
12	Swab	Severe	31.43	33	–	–	NA	+	–
13	Swab	Severe	30.71	31.71	+	–	–	–	–
14	Swab	Mild	38.86	40.28	+	–	–	–	–
15	Swab	Severe	33.86	35.43	+	NA	–	–	–
16	Swab	Mild	39.28	39.85	+	–	NA	–	–
17	Swab	Mild	33.57	35.43	+	–	NA	–	–
18	Swab	Severe	31.86	32·71	+	–	NA	–	–
19	Swab	Severe	37.57	37.86	+	–	NA	NA	–
20	Swab	Mild	28.86	37.71	+	–	NA	NA	NA
21	Serol	Mild	28	39.86	+	–	NA	–	NA
22	Serol	Mild	34.28	36.71	+	–	NA	–	NA

Note: + positive result; – negative result.

Abbreviations: GA_D, gestational age at delivery; GA_S, gestational age at symptoms; NA, not available; Serol: serology.

SARS-CoV-2 AF and/or CB among the 54 neonates tested for SARS-CoV-2 (Table 5). SARS-CoV-2 positivity in AF and/or CB was not associated with neonatal outcomes.

In two neonates (3.7%; 2/54), OPS was positive for SARS-CoV-2, suggesting vertical transmission. These two cases represent 25% (2/8) of the AF and/or CB samples positive for SARS-CoV-2 among the cases for which OPS was examined for SARS-CoV-2 ($N = 54$). For the two cases with possible vertical transmission, SARS-CoV-2 was identified in three compartments, but not in the colostrum, for one; for the other case, the CB and colostrum samples were positive for SARS-CoV-2 (Table 2).

The two neonates with suspected vertical transmission were born at HC and had the following outcomes (Table 2, cases 1 and 2). The first was a female delivered by caesarean section at 33.57 weeks of gestation due to worsening of the maternal COVID-19 condition. The 5-min Apgar score was 9, and the birth weight was 2130 g. OPS samples collected at 48 h of life and repeated at 72 h were positive for SARS-CoV-2. RT-PCR for SARS-CoV-2 was also evaluated on the 17th day of life and was positive, becoming negative on the 22nd day after delivery. Serology performed on the 23rd of life showed IgG positivity. On the third day, the neonate presented decreased oxygen pulse saturation requiring oxygen inhalation until the 14th day of life. The lungs were normal on chest X-ray carried out on the fifth day of life; however, lung ultrasound revealed the presence of coalescent B-lines and subpleural consolidations on the base of the left posterior lung. Additionally, chest computerized tomography scan showed opacities and atelectasis in the right upper lobe and a slightly diffuse increase in the attenuation of the lung parenchyma. Her blood cell counts were normal. Within 22 days, the infant presented enterorrhagia requiring blood transfusion. The infant was discharged from the hospital on the 26th day of life. The newborn was fed formula until the day of hospital discharge.

The second case of positive neonatal OPS was a male born at 38.57 weeks by vaginal delivery with spontaneous labor and rupture of the membranes two hours before birth. The 5-min Apgar score was 9, and the birth weight was 2980 g. The SARS-CoV-2 RT-PCR result using OPS collected on the second day of life was positive, and the test was repeated at 72 h of life, with a positive result. On the seventh day of life, before hospital discharge, OPS testing was repeated, and the result was negative. During the

TABLE 3 Association of maternal COVID-19 status and obstetrics parameters with positive maternal blood RT-PCR for SARS-CoV-2 at delivery (*N* = 109)

Parameters	Maternal blood		
	Positive SARS-CoV-2 RT-PCR (*N* = 16)	Negative SARS-CoV-2 RT-PCR (*N* = 93)	*p*
COVID-19 Symptoms by pregnancy trimester (*N* = 108)			
First	0 (0)	3 (3.3)	0.007[c]
Second	0 (0)	31 (33.7)	
Third	16 (100)	58 (63)	
Hospital admission due to COVID-19	14 (87.5)	66 (71)	0.23[c]
Severe COVID-19[a]	7 (43.8)	33 (35.5)	0.58[c]
Interval COVID-19 symptoms to delivery, days (*N* = 108)	7.5 (2–83)	29 (2–242)	0.002[d]
Interval COVID-19 symptoms to delivery (*N* = 108)			
≤10 days	10 (62.5)	23 (25)	0.006[c]
>10 days	6 (37.5)	69 (75)	
Caesarean section	13 (81.3)	66 (71)	0.55[c]
Gestational age at delivery, weeks	37.2 (31.7–40.3)	38.3 (27.1–41.1)	0.38[d]
BMI (*N* = 107)	29.7 (20.9–43.7)	30.5 (18.7–48.3)	0.56[d]
Prepregnancy morbidity			
Hypertension	0 (0)	14 (15.1)	0.22[c]
Diabetes	2 (12.5)	2 (2.2)	0.10[c]
Other[b]	5 (31.3)	26 (28)	0.77[c]
Obstetric complications			
Preeclampsia	0 (0)	10 (10.8)	0.35[c]
Gestational diabetes	4 (25)	17 (18.3)	0.51[c]

Note: Data are presented as the number (percentage) and median (range).

Abbreviations: BMI, body mass index; RT-PCR, real time polymerase chain reaction.

[a]Severe COVID-19: required oxygen supply or intensive care unit admission.

[b]Other: cardiac disease, lung disease; hypothyroidism; anemia, neurological disorders.

[c]Fisher exact test.

[d]Mann–Whitney test.

hospital stay, the blood cell count and lung ultrasonography were normal. On his third day, the neonate presented asymptomatic sinus bradycardia associated with hypocalcemia. After 3 days of enteral calcium infusion the heart rate and serum calcium levels were normal. The newborn was fed with formula until hospital discharge at 8 days of life.

4 | DISCUSSION

The findings of this prospective study conducted in a single center over a 6-month period demonstrated the following. First, vertical transmission of SARS-CoV-2 from a mother with COVID-19 during pregnancy to their baby is possible. Second, SARS-CoV-2 can be recovered in all maternal biological compartments that may expose the neonate (AF, CB and colostrum). Third, recent infection in the mother is associated with positivity for SARS-CoV-2 in maternal blood and in the AF and/or CB compartments. Fourth, the presence of SARS-CoV-2 RT-PCR positivity in maternal blood at delivery is related to SARS-CoV-2 RT-PCR positivity in AF and/or CB.

The possibility of vertical transmission of SARS-CoV-2 during pregnancy was described previously.[5,10,12–14,18,27] Hitherto, the transplacental transmission of SARS-CoV-2 was demonstrated in only few studies,[14,15,18] with virus positivity in AF, CB and/or placenta. In our study, 3.7% of the neonates tested had positive OPS RT-PCR results for SARS-CoV-2. This result is similar to the findings described in a meta-analysis[15] involving 936 neonates (3.2%), however we observed higher rates of SARS-CoV-2 RT-PCR positivity for AF (13.9% vs. 0%) and CB (6.7% vs. 2.9%). Recently, other studies[6,8,9,28] observed none or only a few cases of vertical transmission, and these conflicting results may be related to the severity of our cohort.

TABLE 4 Association of maternal COVID-19 and obstetrics parameters with positive RT-PCR for SARS-CoV-2 in compartments (amniotic fluid and/or cord blood) at delivery (*N* = 109)

Parameters	Compartments		*p*
	Positive SARS-CoV-2 RT-PCR (*N* = 11)	Negative SARS-CoV-2 RT-PCR (*N* = 98)	
COVID-19 symptoms by pregnancy trimester (*N* = 108)			
First	0 (0)	3 (3.1)	0.33[c]
Second	1 (9.1)	30 (30.9)	
Third	10 (90.9)	64 (66)	
Hospital admission due to COVID-19	10 (90.9)	70 (71.4)	0.28[c]
Severe COVID-19[a]	5 (45.5)	35 (35.7)	0.53[c]
Interval COVID-19 symptoms to delivery, days (*N* = 108)	7 (2-95)	27 (2-242)	0.049[d]
Interval COVID-19 symptoms to delivery (*N* = 108)			
≤10 days	7 (63.6)	26 (26.8)	0.032[c]
>10 days	4 (36.4)	71 (73.2)	
Positive SARS-CoV-2 RT-PCR for the maternal blood at delivery	6 (54.5)	10 (10.2)	0.001[c]
Caesarean section	9 (81.8)	70 (71.4)	0.72[c]
Gestational age at delivery, weeks	38.6 (33.6–40)	37.8 (27.1–41.1)	0.72[d]
BMI (*N* = 107)	29.4 (23.1–46.2)	30.6 (18.7–48.3)	0.47[d]
Prepregnancy diseases			
Hypertension	0 (0)	14 (14.3)	0.35[c]
Diabetes	1 (9.1)	3 (3.1)	0.35[c]
Other[b]	3 (27.3)	28 (28.6)	1.00[c]
Obstetric complications			
Preeclampsia	1 (9.1)	9 (9.2)	1.00[c]
Gestational diabetes	1 (9.1)	20 (20.4)	0.69[c]

Note: Data are presented as the number (percentage) and median (range).

Abbreviations: BMI, body mass index; RT-PCR, real time polymerase chain reaction.

[a]Severe COVID-19: required oxygen supply or intensive care unit admission.

[b]Other: cardiac disease, lung disease; hypothyroidism; anemia, neurological disorders.

[c]Fisher exact test.

[d]Mann–Whitney test.

Furthermore, the paucity of publications and the lack of a standardized definition of vertical transmission of SARS-CoV-2 hamper the comparison with results from previous studies. There is no consensus yet as to refer congenital infection in the presence of a positive PCR for SARS-CoV-2 in CB and AF collected at delivery. Thus, for caution, in the present study the criteria by the WHO[21] were used to classify vertical transmission, which does not consider isolated positive results of CB or AF at delivery as congenital infection. However, we agree with Shah et al.[22] that similar to what is considered for other congenital infections, the presence of the virus in CB or AF should be stated as fetal infection.

Although we believe that the positivity in these samples demonstrate the passage of the virus through the placenta, it does not necessarily reflect a fetal/neonatal compromise, once we had a significant number of positive results in the compartments but only two positive OPS in the neonates. The lower positivity rates of RT-PCR in the neonates may be the result of a balance between the ability of the virus in compromising the fetus and neonates and their immunological response to infection,[21] however more studies are needed to evaluate the significance of these findings.

Despite all the efforts to avoid contamination of the samples during collection, this possibility cannot be completely excluded, and a positive umbilical CB or AF could be a result of maternal contamination. Thus, we believe that the persistence of a positive test on subsequent specimens is critical to differentiate whether there is superficial contamination or actual fetal/neonatal infection.

TABLE 5 Association of neonatal parameters according to SARS-CoV-2 RT-PCR positivity in compartments (amniotic fluid and/or cord blood) in cases in which the neonates were tested for SARS-CoV-2 by oropharyngeal swab ($N = 54$)

Parameters	Compartments		p
	Positive SARS-CoV-2 RT-PCR ($N = 8$)	Negative SARS-CoV-2 RT-PCR ($N = 46$)	
Gestational age at delivery, weeks	38.3 (33.6–40)	35.6 (27.1–41.1)	0.20[b]
Sex			
Female	3 (37.5)	22 (47.8)	0.71[a]
Male	5 (62.5)	24 (52.2)	
Birth weight	3080 (1732–3490)	2490 (680–3870)	0.22[b]
Apgar score at 5 min <7	1 (12.5)	8 (17.4)	1.00[a]
ICU admission	2 (25)	26 (56.5)	0.13[a]
Length of hospital stay, days	5.5 (2–29)	6 (2–190)	0.39[b]
Requiring mechanical ventilation	0 (0)	10 (21.7)	0.33[a]

Note: Data are presented as the number (percentage) and median (range).

Abbreviations: ICU, intensive care unit; RT-PCR, real time polymerase chain reaction.

[a]Fisher exact test;

[b]Mann–Whitney test.

We acknowledge that despite of the finding that the shorter infection-delivery interval is the main factor for vertical transmission this aspect could also be related to mechanical transmission throughout contamination during delivery. However, we took all the precautions during the samples collections and the newborn was immediately separated from the mother soon after birth.

The major strength of this study is that we evaluated a systematic collection of samples that represents exposure of the neonate. Indeed, in the two neonates with positive OPS samples, at least two of the compartments were positive, reinforcing the hypothesis of intrauterine transmission.

Meanwhile, the possibility of a higher number of undiagnosed neonates cannot be excluded, since the sensitivity of RT-PCR of NPS/OPS varies according to time and site of collection.[19,20] We do acknowledge that serial collection of NPS/OPS samples after birth and a wider panel evaluation of multiple biological sites may increase the sensitivity of viral detection and could be helpful to evaluate those cases with positive RT-PCR in AF and CB. Unfortunately, the analysis of all samples collected were made retrospectively, and it was not possible to select the cases in which this strategy should be applicable.

Interestingly, RT-PCR positivity was detected for some AF and/or CB samples, with a more than 4-week interval between maternal symptoms and delivery. Nonetheless, the prolonged presence of the virus is unusual and does not mean that the virus is able to replicate and cause infection, and additional analysis such as viral culture could have clarified this aspect. Considering this finding, it is still reasonable to think that investigation of AF proximate to infection could bring important information about vertical transmission in mothers with COVID-19 diagnosis in the first and second trimester. Performing amniocentesis in those cases could be helpful to understand the possibility of intrauterine transmission in cases with SARS-CoV-2 infection at earlier stages of gestation.

In our sample, we observed two cases with positive colostrum. In one case, CB was also positive, though none of the other tested compartments was positive in the second case. Viral cultures were negative for these two cases, suggesting that SARS-CoV-2 RNA may not represent replication-competent virus in the colostrum. As demonstrated in our study, previous research has also reported 1/18 positive cases of SARS-CoV-2 in the colostrum and maternal milk,[29] and another study found one case (1/11) of SARS-CoV-2 positivity in maternal milk.[14] Despite our finding of SARS-CoV-2 positivity in colostrum samples, it is not possible to assume that mother-to-infant transmission occurs by breastfeeding, and further studies with larger samples are needed.

Our finding that the interval between COVID-19 symptoms and delivery influences the detection of SARS-CoV-2 in AF and CB, in turn increasing potential exposure of the fetus and neonate, has major clinical implications. These implications include (1) the need to reinforce personal protection for pregnant women; (2) whenever possible, delivery should be avoided during the acute phase of COVID-19 infection; and (3) molecular tests to evaluate the possibility of SARS-CoV-2 infection should be performed for all neonates born to mothers with a recent diagnosis of COVID-19.

5 | CONCLUSION

In conclusion, our study supports the possibility of vertical transmission of SARS-CoV-2 from infected symptomatic mothers to their

infants, particularly if infection occurs close to delivery. Therefore, our data suggest that special care is needed in pregnant women with COVID-19 and that whenever possible, delivery in the acute phase of the disease should be avoided.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

ETHICAL APPROVAL

The study protocol was approved by the ethics committees of both hospitals (CAAE: 30270820.3.0000.0068, approved on April 11th, 2020) and was registered at ClinicalTrial.gov (NCT04647994). All participants provided informed consent before participating in the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Mariane de Fátima Yukie Maeda https://orcid.org/0000-0003-3977-4954

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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