

1 **Vertical transmission of SARS-CoV2 during pregnancy: prospective cohort study**

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29 **Funding:** CAPES (88881.504727/2020-01) and by the European Union's Horizon 2020
30 Research and Innovation Programme under ZIKAlliance Grant Agreement No 734548. The
31 funders had no role in the study design, data collection and analysis, decision to publish, or
32 preparation of the manuscript.

33

34 **Short title: Vertical transmission of SARS-CoV-2**

35

36 **Acknowledgements:** We acknowledge all members of the HC-FMUSP-Obstetric COVID19 Study
37 Group: Alan Garcia da Silva; Aline Scalisse Bassi; Amanda Wictky Fabri; Ana Claudia Rodrigues
38 Lopes Amaral de Souza; Ana Claudia Silva Farche; Ana Maria Kondo Igai; Ana Maria da Silva Sousa
39 Oliveira; Adriana Lippi Waissman; Carlos Eduardo do Nascimento Martins; Cristiane de Freitas
40 Paganoti; Danielle Rodrigues Domingues; Fernanda Cristina Ferreira Mikami; Fernanda Spadotto
41 Baptista; Jacqueline Kobayashi Cippiciani; Jéssica Gorrão Lopes Albertini; Joelma Queiroz de
42 Andrade; Juliana Ikeda Niigaki, Lucinda Cristina Pereira; Marco Aurélio Knippel Galletta; Mariana
43 Yumi Miyadahira, Mariana Vieira Barbosa; Monica Fairbanks de Barros; Nilton Hideto Takiuti;
44 Sckarlet Ernandes Biancolin Garavazzo; Silvio Martinelli; Tiago Pedromonico Arrym; Ursula Trovato
45 Gomez; Veridiana Freire Franco.

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54

55 **Précis**

56 Vertical transmission is possible in pregnant women with SARS-CoV-2 infection, and a shorter
57 interval between symptoms to delivery is associated with mother-to-child transmission.

58

59 Abstract

60 **Objective:** To evaluate the potential for and risk factors of SARS-CoV-2 vertical transmission.

61 **Methods:** A cohort of pregnant women with confirmed coronavirus disease 2019 (COVID-19)
62 diagnosis (positive swab and/or serology) in whom reverse transcription-polymerase chain
63 reaction (RT-PCR) for SARS-CoV-2 was performed at delivery using maternal serum and at
64 least one of the following biological samples: cord blood (CB), amniotic fluid (AF) or
65 colostrum and/or oropharyngeal swab (OPS) of the neonate, with the samples being collected
66 at 48 hours after birth. The association of maternal and obstetrics parameters with maternal
67 blood positivity and AF and/or CB at delivery and the influence of SARS-CoV-2 positivity in
68 AF and/or CB on neonatal outcomes were investigated.

69 **Results:** A total of 109 pregnant women were included. Positive RT-PCR for SARS-CoV-2
70 was observed in 14.7% (16/109) of maternal blood samples, 13.9% (6/43) of AF samples,
71 6.7% (7/105) of CB samples, 2.1% (2/97) of colostrum samples and 3.7% (2/54) of neonatal
72 OPS samples. The duration of the interval between COVID-19 symptoms and delivery was
73 inversely associated with SARS-CoV-2 positivity in the maternal blood ($p= 0.002$) and in the
74 AF and/or CB ($p= 0.049$). Moreover, there was an association between SARS-CoV-2 positivity
75 in maternal blood and SARS-CoV-2 positivity in AF and/or CB ($p= 0.001$). In turn, SARS-
76 CoV-2 positivity in AF and/or CB was associated with OPS positivity in neonates ($p= 0.020$).
77 **Conclusion:** The findings support that vertical transmission can occur in pregnant women with
78 COVID-19 and that a shorter interval between maternal symptoms and delivery is an
79 influencing factor.

80

81 Introduction

82 Although the burden of COVID-19 for individuals with particular conditions, such as
83 chronic diseases, has been described to some extent¹, the impact of disease and/or the virus on

84 pregnant women and their offspring is not very well documented. In addition, it is not well
85 known whether the disease at an acute stage or severe disease around the time of delivery have
86 different impacts on pregnancy outcomes and vertical transmission.²⁻⁹

87 Several recent reports of vertical transmission have been published, mostly based on
88 case reports, case series, or meta-analysis and reviews of such series. Conversely, few studies
89 have systematically investigated the presence of SARS-COV-2 in biological samples such as
90 cord blood (CB), placental tissue, and amniotic fluid (AF) to support the role of vertical
91 transmission.^{5,8,10-13} Moreover, the results of these reports¹⁴⁻¹⁷ have been inconsistent, which
92 may be due to the timing of sample collection (during the acute or post-recovery phase of
93 maternal COVID-19), type of assays used to detect the presence of the virus, and lack of test
94 accuracy.¹⁸⁻¹⁹

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

103

104 **Material and Methods**

105 This analysis of vertical transmission is one of the aims of a major cohort study,
106 “exploratory study on COVID-19 in pregnant women,” which began on April 12th at Hospital
107 das Clinicas (HC-FMUSP) and Hospital Universitario (HU-USP), Sao Paulo University and is

108 ongoing. The exploratory study also included estimating the seroprevalence of SARS-CoV-2
109 at delivery at HU.

110 When the COVID-19 pandemic started in Sao Paulo, our institution organized the
111 Hospital das Clinicas (HC) as a COVID-19 hospital and the Hospital Universitario (HU) as a
112 non-COVID-19 hospital. Therefore, pregnant women with COVID-19 or flu symptoms or
113 contact with a SARS-CoV-2-positive person would be seen at HC; others would be seen at HU.
114 In addition, at HU, a triage system at the hospital entrance was established; pregnant women
115 with COVID-19 or flu symptoms or contact with a SARS-CoV-2-positive person were referred
116 immediately to HC at any time during pregnancy, delivery or puerperium. Patients seen at HC
117 were only allowed to follow the antenatal or puerperium at HU after 14 days of quarantine and
118 without symptoms.

119 For the present investigation, pregnant women who fulfilled the following inclusion
120 criteria were selected: 1) singleton pregnancies with live fetuses with a diagnosis of COVID-
121 19 during pregnancy or at delivery by RT-PCR using a nasopharyngeal swab (NPS) or
122 serology; 2) COVID-19 or flu-like symptoms during pregnancy or at delivery; and 3)
123 investigation of SARS-CoV-2 by RT-PCR using maternal blood and at least one biological
124 sample at delivery (CB, AF) or after birth (colostrum or oropharyngeal swab (OPS) from the
125 neonate).

126 All pregnant women admitted to HC were investigated for SARS-CoV-2 infection by
127 molecular testing (RT-PCR) using samples collected from the respiratory tract (nasopharynx
128 and/or trachea) from the 3rd day of symptoms. In cases with negative results, NPS testing was
129 repeated, or SARS-CoV-2 serology was performed after the 8th day of symptoms. The
130 following symptoms were considered COVID-19 symptoms: fever or chills, cough, dyspnea,
131 fatigue, myalgia, sore throat, headache, congestion, or runny nose; loss of taste or smell;
132 diarrhea. In addition, any flu-like symptoms were considered COVID-19 symptoms until
133 proven otherwise.

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

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

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

157 For neonates born at HC, a swab was collected from the oropharynx and trachea (if the
158 newborn was intubated), or two samples from the oropharynx were collected at 48 hours after
159 delivery for SARS-CoV-2 RT-PCR analysis. In cases of any positive test for the neonate, an

160 additional sample was obtained at 72 hours after delivery and tested to exclude false-positive
161 results. Neonates born at HU were not subjected to SARS-CoV-2 investigation, as the mothers
162 were not in the acute phase of the disease, and breastfeeding was allowed.

163 **Laboratory methods**

164 Nucleic acid was extracted from 140 μ L of clinical samples using a QIAmp Viral RNA
165 mini kit (Qiagen, Germany), eluted in 60 μ L and stored at -80°C until processing. Detection of
166 viral RNA was performed using a qualitative RT-PCR - RealStar SARS-CoV-2 RT-PCR kit
167 1.0 RUO (Altona Diagnostic, Germany) according to the manufacturer's instructions. The
168 reaction targets the β -coronavirus E gene and SARS-CoV-2 S gene. The assay was performed
169 using a LightCycler 96 Instrument (Roche, Germany).

170 Samples were considered positive when the cycle threshold (*Ct*) value was ≤ 40 for at
171 least one of the targets. The assay was evaluated with an independent validation by Visseaux
172 et al.²⁰, showing high sensitivity and specificity comparable with protocols currently
173 recommended by the Health Organization (WHO).

174 A serological test (IgG/IgM antibodies) was performed using the Wondfo One Step
175 COVID-19 test (Guangzhou Wondfo Biotech, China). The test was performed using 10 μ L of
176 serum pipetted into the sample cavity of the test device, after which 80 μ L was added to the
177 cavity below the sample cavity. The result was read in 15 minutes by three people that had
178 received appropriate training. The color change was compared to the assay standard.²¹

179 For viral culture, Vero cells (ATCC CCL-81) were used as previously described.²²⁻²⁴
180 Vero CCL81 cells were cultured in Dulbecco minimal essential medium supplemented with
181 heat-inactivated fetal bovine serum (10%) and antibiotics/antimycotics (Cultilab, Campinas,
182 Brazil).

183 For virus isolation, samples were inoculated in Vero cell culture in plastic bottles (jet
184 biofilm, 12.5cm² area, 25mL capacity) immediately after processing. The inoculated cultures

185 were grown in a 37°C incubator in an atmosphere of 5% CO₂. The cell cultures were maintained
186 for at least two weeks and observed daily for evidence of the cytopathic effect.

187 At least two subcultures were performed weekly. Presumptive detection of virus in
188 supernatants showing cytopathic effect was investigated using an inverted microscope (Nikon,
189 Japan) and confirmed by specific RT-PCR as described above.

190 Quantitative continuous variables are presented as means and standard deviations (SD)
191 or medians and minimum and maximum values. Categorical variables are presented as absolute
192 frequencies and percentages. Comparison between groups was performed using Fisher's exact
193 test for unpaired samples with normal distribution; when the distribution was not normal, the
194 Mann-Whitney U nonparametric test was employed. Differences were considered significant
195 when the p-value was less than 0.05. The data were analyzed using Statistical Package for the
196 Social Sciences (SPSS version 20 IBM, Armonk, NY, USA).

197 The study protocol was approved by the ethics committees of both hospitals (CAAE:
198 30270820.3.0000.0068) and was registered at ClinicalTrial.gov (NCT04647994). All
199 participants provided informed consent before participating in the study.

200

201 **Results**

202 Between 12th April and 30th September, 1044 pregnant women were admitted at HC and HU,
203 and 595 pregnant women being assessed; 109 fulfilled the inclusion criteria for the present
204 analysis (**Figure 1**). Details regarding the demographics, obstetrics and clinical characteristics
205 of the participants are presented in **Table 1**. For 108 cases, the median interval between
206 COVID-19 symptoms and delivery was 23.5 days (7.2-76.2). In one case, the patient
207 experienced COVID-19 symptoms during pregnancy but was not able to provide the timing;
208 therefore, this case was not included in the analysis. The majority of patients (80/109, 73.4%)
209 were admitted to the hospital due to COVID-19, and 36.7% (40/109) had severe disease.

210 Rates of RT-PCR positivity for SARS-CoV-2 in biological samples collected at
211 delivery were 14.7% (16/109) for maternal blood, 13.9% (6/43) for AF and 6.7% (7/105) for
212 CB. Overall, there were some difficulties regarding the collection of AF due to the severity of
213 some cases and the need to ascertain that the samples were not contaminated with maternal
214 fluids during delivery, which contributed to the small number of AF samples collected. In total,
215 2.1% (2/97) of the maternal colostrum samples were positive for SARS-CoV-2 by RT-PCR.
216 Viral culture was performed in these two positive cases and did not show viral replication,
217 which may be due to the high fat composition of colostrum, which can render this analysis
218 difficult.

219 Distributions of SARS-CoV-2 status in the investigated compartments are presented in
220 **Table 2**. Details of the cases with at least one positive sample for SARS-CoV-2 are described
221 in **Table 3**.

222 All pregnant women with positive SARS-CoV-2 RT-PCR results for maternal blood at
223 delivery presented COVID-19 symptoms in the third trimester, with a significant difference
224 ($p= 0.007$; **Table 4**). In addition, a significantly shorter interval between the occurrence of
225 COVID-19 symptoms and delivery (7.5 vs 29 days, $p= 0.002$) was observed in cases of SARS-
226 CoV-2 positivity in blood, and this result was also observed when considering intervals ≤ 10
227 or > 10 days ($p= 0.006$; **Table 4**). Conversely, no influence of other maternal, COVID-19-
228 related, or obstetric factors was observed (**Table 4**).

229 **Table 5** provides the associations of maternal COVID-19 and obstetrics parameters
230 with SARS-CoV-2 RT-PCR positivity in AF and/or CB at delivery. The interval between the
231 onset of COVID-19 symptoms and delivery was associated with positive SARS-CoV-2 RT-
232 PCR results in AF and/or CB at delivery (7 vs 27 days, $p= 0.049$), which was also observed for
233 intervals ≤ 10 or > 10 days ($p= 0.032$; **Table 5**). In addition, SARS-CoV-2 RT-PCR positivity
234 in maternal serum at delivery was associated with SARS-CoV-2 RT-PCR positivity in AF

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

237 To investigate whether a positive SARS-CoV-2 RT-PCR result in AF and/or CB
238 influences neonatal outcome, we analyzed the association between neonatal parameters and
239 positive and negative SARS-CoV-2 AF and/or CB among the 54 neonates tested for SARS-
240 CoV-2 using OPS (**Table 6**). SARS-CoV-2 positivity in AF and/or CB was only associated
241 with OPS positivity in the neonate (2 vs 0; p= 0.020).

242 In two neonates (3.7%; 2/54), OPS was positive for SARS-CoV-2, suggesting vertical
243 transmission. These two cases represent 25% (2/8) of the AF and/or CB samples positive for
244 SARS-CoV-2 among the cases for which OPS was examined for SARS-CoV-2 (n= 54). For
245 the two cases with suspected vertical transmission, SARS-CoV-2 was identified in three
246 compartments, but not in the colostrum, for one; for the other case, the CB and colostrum
247 samples were positive for SARS-CoV-2 (**Table 3**).

248 The two neonates with suspected vertical transmission (**Table 3**) were born at HC and
249 had the following outcomes. The first was a female delivered by caesarean section at 33.57
250 weeks of gestation due to worsening of the maternal COVID-19 condition. The five-minute
251 Apgar score was 9, and the birth weight was 2130 grams. OPS samples collected at 48 hours
252 of life and repeated at 72 hours were positive for SARS-CoV-2. RT-PCR for SARS-CoV-2
253 was also evaluated on the 17th day of life and was positive, becoming negative on the 22nd day
254 after delivery. Serology performed on the 23rd of life showed IgG positivity. On the 3rd day, the
255 neonate presented decreased oxygen pulse saturation requiring oxygen inhalation until the 14th
256 day of life. The lungs were normal on chest X-ray carried out on the 5th day of life; however,
257 lung ultrasound revealed the presence of coalescent B-lines and subpleural consolidations on
258 the base of the left posterior lung. Additionally, chest computerized tomography (CT) scan
259 showed opacities and atelectasis in the right upper lobe and a slightly diffuse increase in the
260 attenuation of the lung parenchyma. Her blood cell counts were normal. Within 22 days, the

261 infant presented enterorrhagia requiring blood transfusion. The infant was discharged from the
262 hospital on the 26th day of life. The newborn was fed formula until the day of hospital discharge.

263 The second case of positive neonatal OPS was a male born at 38·57 weeks by vaginal
264 delivery with spontaneous labor and rupture of the membranes two hours before birth. The
265 five-minute Apgar score was 9, and the birth weight was 2980 grams. The SARS-CoV-2 RT-
266 PCR result using OPS collected on the second day of life was positive, and the test was repeated
267 at 72 hours of life, with a positive result. On the 7th day of life, before hospital discharge, OPS
268 testing was repeated, and the result was negative. During the hospital stay, the blood cell count
269 and lung ultrasonography were normal. On his 3rd day, the neonate presented asymptomatic
270 sinus bradycardia associated with hypocalcemia. After three days of enteral calcium infusion
271 the heart rate and serum calcium levels were normal. The newborn was fed with formula until
272 hospital discharge at 8 days of life.

273

274 **Discussion**

275 The findings of this prospective study conducted in a single center over a six-month
276 period demonstrated the following. First, vertical transmission of SARS-CoV-2 from a mother
277 with COVID-19 during pregnancy to their baby is possible. Second, SARS-CoV-2 can be
278 recovered in all maternal biological compartments that may expose the neonate (AF, CB and
279 colostrum). Third, recent infection in the mother is associated with positivity for SARS-CoV-
280 2 in maternal blood and in the AF and/or CB compartments. Fourth, the presence of SARS-
281 CoV-2 RT-PCR positivity in maternal blood at delivery is related to SARS-CoV-2 RT-PCR
282 positivity in AF and/or CB, which in turn influences the finding of SARS-CoV-2 positivity in
283 the neonate.

284 The possibility of vertical transmission of SARS-CoV-2 during pregnancy has been
285 described previously.¹⁰ In our study, 3.7% of the neonates tested had positive OPS RT-PCR

286 results for SARS-CoV-2, similar to findings described in a recent meta-analysis¹¹ involving
287 936 neonates (3.2%). However, we observed higher rates of SARS-CoV-2 RT-PCR positivity
288 for AF (13.9% vs 0%) and CB (6.7% vs 2.9%) than reported in this previous meta-analysis.

289 Interestingly, RT-PCR positivity was detected for some AF and/or CB samples, with a
290 more than four-week interval between maternal symptoms and delivery. Nonetheless, the
291 prolonged presence of the virus is unusual and does not mean that the virus is able to replicate
292 and cause infection. To clarify this issue, it is necessary to cultivate the virus to determine viral
293 replication in these samples.

294 In our sample, we observed SARS-CoV-2 RT-PCR positivity in 2.1% of colostrum
295 samples. In one case, CB was also positive, though none of the other tested compartments was
296 positive in the other case. Viral cultures were negative for these two cases, suggesting that
297 SARS-CoV-2 RNA may not represent replication-competent virus in the colostrum. As
298 demonstrated in our study, previous research has also reported 1/18 positive cases of SARS-
299 CoV-2 in the colostrum and maternal milk,²⁵ and another recent study found one case (1/11) of
300 SARS-CoV-2 positivity in maternal milk collected on the 5th day after delivery.¹⁰

301 Our finding that the interval between COVID-19 symptoms and delivery influences the
302 detection of SARS-CoV-2 in AF and CB, in turn increasing potential exposure of the fetus and
303 neonate, has major clinical implications. These implications include (i) the need to reinforce
304 personal protection for pregnant women mainly in the third trimester of pregnancy; (ii)
305 whenever possible, delivery should be avoided during the acute phase of COVID-19 infection;
306 and (iii) molecular tests (OPS or serum RT-PCR) to evaluate the possibility of SARS-CoV-2
307 infection should be performed for all neonates born to mothers with a recent diagnosis of
308 COVID-19.

309 Despite our finding of SARS-CoV-2 positivity in colostrum samples, it is not possible
310 to assume that mother-to-infant transmission occurs by breastfeeding, and further studies with
311 larger samples are needed.

312 All but one case of maternal sample positivity occurred among pregnant women who
313 developed COVID-19 in the third trimester. The two neonates with positive OPS samples
314 presented mild symptoms after delivery. It seems that SARS-COV-2 can present similar
315 characteristics to other pathogens, such as cytomegalovirus and toxoplasma, whereby
316 transplacental passage tends to increase with gestational age but with lower fetal/neonatal
317 compromise.²⁶ Nevertheless, our study only included three women with COVID-19 in the first
318 trimester. Further cohort studies including pregnant women who have acquired SARS-CoV-2
319 at earlier stages of pregnancy are required to evaluate the true effects of infection by this virus
320 on vertical transmission.

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

325 The main limitation of the study is that we did not more fully ascertain the infection
326 status of the neonates by testing their serum for SARS-CoV-2 by RT PCR or performing IgM
327 serology, which may have provided additional cases of vertical transmission, as it is known
328 that NPS/OPS may lack sensitivity and that a positive result may be secondary to intrapartum
329 contamination.¹⁸ At this stage of the epidemic, the optimal test to determine infection status in
330 the neonate remains unclear.²⁷ We acknowledge that a wider panel evaluation of multiple
331 biological sites may have increased the sensitivity of viral detection.

332 In conclusion, our study confirms the possibility of vertical transmission of SARS-
333 CoV-2 from infected symptomatic mothers to their infants, particularly if infection occurs close

334 to delivery. Therefore, our data suggest that special care is needed in pregnant women with
335 COVID-19 in the third trimester and that whenever possible, delivery in the acute phase of the
336 disease should be avoided.

337

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448 **Table 1.** Study population demographic, clinical, obstetrical, and neonatal characteristics.

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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*	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 (3-12)
Required oxygen supply	38 (34.9)
ICU due to COVID-19	25 (22.9)
Severe COVID-19	40 (36.7)
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 minutes <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

450 *Other: cardiac disease, lung disease, hypothyroidism, anemia, neurological disorders. Severe COVID-19:
 451 required oxygen supply or ICU admission. ICU: intensive care unit; NPS: nasopharyngeal swab; SD: standard
 452 deviation; IQR: interquartile range
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460 **Table 2.** Distribution of SARS-CoV-2 PCR status in compartments (maternal blood, amniotic fluid,
 461 umbilical cord blood and maternal colostrum) and oropharyngeal swabs of the neonate (N= 109).
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Number of cases	Maternal blood	Cord blood	Amniotic Fluid	Colostrum	Neonate
1	+	+	+	-	+
1	-	+	-	+	+
2	+	+	NA	-	-
2	-	+	NA	-	-
1	-	+	+	-	NA
1	+	-	+	-	-
1	+	-	+	NA	-
2	+	NA	1 (+),1(-)	-	-
1	-	-	+	-	NA
1	-	-	NA	+	-
5	+	-	NA	-	3 (-), 2 (NA)
2	+	-	NA	NA	1 (-), 1 (NA)
2	+	-	-	-	-
39	-	-	9 (-), 30 (NA)	-	NA
34	-	-	16 (-), 18 (NA)	-	-
5	-	-	-	NA	NA
2	-	-	-	NA	-
2	-	-	NA	NA	NA
2	-	-	NA	NA	NA
1	-	-	NA	NA	-
1	-	NA	-	-	-
1	-	NA	NA	-	NA

463 NA= not available; + positive result; - negative result.

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Table 3. Details of cases with at least one positive biological sample for SARS-CoV-2 at delivery.

Case	Diag	COVI D-19	GA_S	GA_D	Maternal blood RT-PCR	Cord blood RT-PCR	Amniotic fluid RT-PCR	Colostrum RT-PCR	Neonate Swab
1	Swab	Severe	32.86	33.57	Ct 33.52 Gene E; Ct 31.72 Gene S	Ct 32.44 Gene E; Ct 29.82 Gene S	Ct 29.08 Gene E	Neg	Pos
2	Swab	Mild	38.14	38.57	Neg	Ct 21.43 Gene E; Ct 20.53 Gene S	Neg	Ct 32.6 Gene E; Ct 31.36 Gene S	Pos
3	Swab	Mild	38.43	39.43	Neg	Ct 30.22, Gene E	NA	Neg	Neg
4	Swab	Mild	29	38.43	Neg	Ct 36.87 Gene E; Ct 36.04 Gene S	Ct 35.27 Gene E; Ct 33.65 Gene S	Neg	NA
5	Swab	Mild	30.14	33.71	Ct 30.70, Gene E; Ct 29.55 Gene S	Ct 26.90, Gene E; Ct 25.46 Gene S	NA	Neg	Neg
6	Swab	Severe	25.57	39.14	Neg	Ct 37.75 Gene E	NA	Neg	NA
7	Swab	Severe	38.28	39.14	Ct 35.17, Gene E	Ct 34.34, Gene E	NA	Neg	Neg
8	Swab	Severe	34	35.14	Ct 37.43, Gene E	Neg	Ct 29.59, Gene E; Ct 28.03 Gene S	Neg	Neg
9	Swab	Severe	30.14	39.86	Neg	Neg	Ct 37.90 Gene E	Neg	NA
10	Swab	Mild	37.71	38	Ct 36.24, Gene E	Neg	Ct 35.44, Gene E; Ct 29.36 Gene S	NA	Neg
11	Swab	Mild	39.28	40	Ct 37.52, Gene E	NA	Ct 29.84, Gene E; Ct 28.37 Gene S	Neg	Neg
12	Swab	Severe	31.43	33	Neg	Neg	NA	Ct 30.26, Gene E; Ct 29.49 Gene S	Neg

13	Swab	Severe	30.71	31.71	Ct 34.42 Gene E	Neg	Neg	Neg	Neg
14	Swab	Mild	38.86	40.28	Ct 37.00, Gene E	Neg	Neg	Neg	Neg
15	Swab	Severe	33.86	35.43	Ct 34.85, Gene E	NA	Neg	Neg	Neg
16	Swab	Mild	39.28	39.85	Ct 34.02, Gene E	Neg	NA	Neg	Neg
17	Swab	Mild	33.57	35.43	Ct 28.45 Gene E; Ct 27.94 Gene S; Ct 28.38 Gene E	Neg	NA	Neg	Neg
18	Swab	Severe	31.86	32.71	Ct 31.47 Gene S; Ct 30.70 Gene E	Neg	NA	Neg	Neg
19	Swab	Severe	37.57	37.86	Ct 33.36, Gene E	Neg	NA	NA	Neg
20	Swab	Mild	28.86	37.71	Ct 35.06 Gene E	Neg	NP	NA	NA
21	Serol	Mild	28	39.86	Ct 35.61, Gene E	Neg	NA	Neg	NA
22	Serol	Mild	34.28	36.71	Ct 36.85 Gene E	Neg	NA	Neg	NA

468 Ct: cycle threshold; Diag: diagnosis method; Serol: serology; GA_S: gestational age at symptoms; GA_D:
469 gestational age at delivery; Neg: negative; Pos: positive; NA: not available

470 **Table 4.** Association of maternal COVID-19 status and obstetrics parameters with positive maternal
 471 blood RT-PCR for SARS-CoV-2 at delivery (N= 109).
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Parameters	Maternal blood		P
	Positive SARS-CoV-2 RT-PCR (N= 16)	Negative SARS-CoV-2 RT-PCR (N= 93)	
COVID-19 Symptoms by pregnancy trimester (N=108)			
First	0 (0)	3 (3.3)	0.007*
Second	0 (0)	31(33.7)	
Third	16 (100)	58 (63)	
Hospital admission due to COVID-19	14 (87.5)	66 (71)	0.23*
Severe COVID-19	7 (43.8)	33 (35.5)	0.58*
Interval COVID-19 symptoms to delivery, days (N=108)	7.5 (2-83)	29 (2-242)	0.002†
Interval COVID-19 symptoms to delivery (N=108)			
≤ 10 days	10 (62.5)	23 (25)	0.006*
> 10 days	6 (37.5)	69 (75)	
Caesarean section	13 (81.3)	66 (71)	0.55*
Gestational age at delivery, weeks	37.2 (31.7-40.3)	38.3 (27.1-41.1)	0.38†
BMI (N=107)	29.7 (20.9-43.7)	30.5 (18.7-48.3)	0.56†
Pregnancy morbidity			
Hypertension	0 (0)	14 (15.1)	0.22*
Diabetes	2 (12.5)	2 (2.2)	0.10*
Other	5 (31.3)	26 (28)	0.77*
Obstetric complications			
Preeclampsia	0 (0)	10 (10.8)	0.35*
Gestational diabetes	4 (25)	17 (18.3)	0.51*

473 Data are presented as the number (percentage) and median (range).
 474 Severe COVID-19: required oxygen supply or ICU admission. BMI: body mass index; Other: cardiac disease,
 475 lung disease; hypothyroidism; anemia, neurological disorders.
 476 * Fisher exact test; † Mann-Whitney test.
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Table 5. 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*
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*
Severe COVID-19	5 (45.5)	35 (35.7)	0.53*
Interval COVID-19 symptoms to delivery, days (N= 108)	7 (2-95)	27 (2-242)	0.049 †
Interval COVID-19 symptoms to delivery (N= 108)			
≤ 10 days	7 (63.6)	26 (26.8)	0.032
> 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 *
Caesarean section	9 (81.8)	70 (71.4)	0.72*
Gestational age at delivery, weeks	38.6 (33.6-40)	37.8 (27.1-41.1)	0.72†
BMI (N= 107)	29.4 (23.1-46.2)	30.6 (18.7-48.3)	0.47†
Pregnancy diseases			
Hypertension	0 (0)	14 (14.3)	0.35*
Diabetes	1 (9.1)	3 (3.1)	0.35*
Other*	3 (27.3)	28 (28.6)	1.00*
Obstetric complications			
Preeclampsia	1 (9.1)	9 (9.2)	1.00*
Gestational diabetes	1 (9.1)	20 (20.4)	0.69*

Data presented as the number (percentage); median (range).
 Severe COVID-19: required oxygen supply or ICU admission; BMI: body mass index; Other: cardiac disease, lung disease; hypothyroidism; anemia, neurological disorders.
 * Fisher exact test; † Mann-Whitney test.

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499 **Table 6.** Association of neonatal parameters according to SARS-CoV-2 RT-PCR positivity in
500 compartments (amniotic fluid and/or cord blood) in cases in which the neonates were tested for
501 SARS-CoV-2 by oropharyngeal swab (N= 54).

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Parameters	Compartments		p
	Positive SARS-CoV-2	Negative SARS-CoV-2	
	RT-PCR (n= 8)	RT-PCR (n= 46)	
Swab positivity for SARS-CoV-2	2 (25)	0 (0)	0.02*
Gestational age at delivery, weeks	38.3 (33.6-40)	35.6 (27.1-41.1)	0.20†
Sex			
Female	3 (37.5)	22 (47.8)	0.71*
Male	5 (62.5)	24 (52.2)	
Birth weight	3080 (1732-3490)	2490 (680.3870)	0.22†
Apgar score at 5 minutes < 7	1 (12.5)	8 (17.4)	1.00*
ICU admission	2 (25)	26 (56.5)	0.13*
Length of hospital stay, days	5.5 (2-29)	6 (2-190)	0.39†
Requiring mechanical ventilation	0 (0)	10 (21.7)	0.33*

503 Data are presented as the number (percentage) and median (range).
504 ICU: intensive care unit * Fisher exact test; † Mann-Whitney test.
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506 Figure legend: Flow-chart of the study population. HU: Hospital Universitario, HC:
507 Hospital das Clinicas.