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

Mariane de Fátima Yukie Maeda, MD[1]

Maria de Lourdes Brizot, PhD[1]

Maria Augusta B. C. Gibelli, MD[2]

Silvia Maria Ibidi, PhD[2,3]

Werther Brunow de Carvalho, PhD[2,3]

Mara Sandra Hoshida, PhD[1]

Clarisse Martins Machado, PhD[4]

Ester Cerdeira Sabino, PhD[5]

Lea Campos de Oliveira da Silva, PhD[6]

Thomas Jaenisch, PhD[7,8]

Maria Cassia Mendes-Correa, PhD[4]

Philippe Mayaud, MD[9]

Rossana Pulcineli Vieira Francisco, PhD[1,3]

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
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.
7. Center for Global Health, Colorado School of Public Health, Aurora, Colorado, USA
8. Heidelberg Institute for Global Health (HIGH), Heidelberg University Hospital, Germany
9. Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, UK

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

Short title: Vertical transmission of SARS-CoV-2

Acknowledgements: We acknowledge all members of the HC-FMUSP-Obstetric COVID19 Study Group: Alan Garcia da Silva; 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; Adriana Lippi Waissman; 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, Lucinda Cristina Pereira; 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.

Corresponding author:

Rossana Pulcineli 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, Brazil, CEP 05403 000

Phone: ++55 11 2661 6209 Fax ++55 11 2661 6445

e-mail: rossana.francisco@hc.fm.usp.br

Précis

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

Abstract

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

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

Results: A total of 109 pregnant women were included. Positive RT-PCR for SARS-CoV-2 was observed in 14·7% (16/109) of maternal blood samples, 13·9% (6/43) of AF samples, 6·7% (7/105) of CB samples, 2·1% (2/97) of colostrum samples and 3.7% (2/54) of neonatal OPS samples. The duration of the interval between COVID-19 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). Moreover, there was an association between SARS-CoV-2 positivity in maternal blood and SARS-CoV-2 positivity in AF and/or CB (p= 0·001). In turn, SARS-CoV-2 positivity in AF and/or CB was associated with OPS positivity in neonates (p= 0·020).

Conclusion: The findings support that vertical transmission can occur in pregnant women with COVID-19 and that a shorter interval between maternal symptoms and delivery is an influencing factor.

Introduction

Although the burden of 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.[2-9]

Several recent reports of vertical transmission have been published, mostly based on case reports, case series, or meta-analysis and reviews of such series. 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.[5,8,10-13] Moreover, the results of these reports[14-17] 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, and lack of test accuracy.[18-19]

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 potential exposure to maternal infection and in the neonate to ascertain 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.

Material and 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-FMUSP) and Hospital Universitario (HU-USP), Sao 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 Sao Paulo, our institution organized the Hospital das Clinicas (HC) as a COVID-19 hospital and the Hospital Universitario (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 RT-PCR using a nasopharyngeal swab (NPS) or serology; 2) COVID-19 or flu-like symptoms during pregnancy or at delivery; and 3) 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 pregnant women admitted to HC were investigated for SARS-CoV-2 infection by molecular testing (RT-PCR) using samples collected from the respiratory tract (nasopharynx and/or trachea) from the 3rd day of symptoms. In cases with negative results, NPS testing was repeated, or SARS-CoV-2 serology was performed after the 8th day of symptoms. 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. In addition, any flu-like symptoms were considered COVID-19 symptoms until proven otherwise.

At HC, pregnant women with flu symptoms were evaluated by our clinical staff and hospitalized if they presented any of the following situations: (i) need for clinical support or (ii) 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, AF, and the placenta. Maternal blood samples were collected through venipuncture immediately before delivery. Cord artery or vein blood samples (5 to 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. Within 48 hours 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 hours 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 hours 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 in the acute phase of the disease, and breastfeeding was allowed.

Laboratory methods

Nucleic acid was extracted from 140 µL of clinical samples using a QIAmp Viral RNA mini kit (Qiagen, Germany), 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, Germany) 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, Germany).

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

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

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

For virus isolation, samples were inoculated in Vero cell culture in plastic bottles (jet biofilm, 12.5cm^2 area, 25mL 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 two 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, Japan) and confirmed by specific RT-PCR as described above.

Quantitative continuous variables are presented as means and standard deviations (SD) 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, Armonk, NY, USA).

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

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 the analysis. The majority of patients (80/109, 73·4%) were admitted to the hospital due to COVID-19, and 36·7% (40/109) had severe disease.

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 2**. Details of the cases with at least one positive sample for SARS-CoV-2 are described in **Table 3**.

All pregnant women with positive SARS-CoV-2 RT-PCR results for maternal blood at delivery presented COVID-19 symptoms in the third trimester, with a significant difference (p= 0·007; **Table 4**). 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 blood, and this result was also observed when considering intervals ≤ 10 or > 10 days (p= 0·006; **Table 4**). Conversely, no influence of other maternal, COVID-19-related, or obstetric factors was observed (**Table 4**).

Table 5 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 5)**. In addition, SARS-CoV-2 RT-PCR positivity in maternal serum at delivery was associated with SARS-CoV-2 RT-PCR 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 SARS-CoV-2 AF and/or CB among the 54 neonates tested for SARS-CoV-2 using OPS (**Table 6**). SARS-CoV-2 positivity in AF and/or CB was only associated with OPS positivity in the neonate (2 *vs* 0; p= 0·020).

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 suspected 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 3**).

The two neonates with suspected vertical transmission (**Table 3**) were born at HC and had the following outcomes. 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 five-minute Apgar score was 9, and the birth weight was 2130 grams. OPS samples collected at 48 hours of life and repeated at 72 hours 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 3rd 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 5th 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 (CT) 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 five-minute Apgar score was 9, and the birth weight was 2980 grams. 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 hours of life, with a positive result. On the 7th day of life, before hospital discharge, OPS testing was repeated, and the result was negative. During the hospital stay, the blood cell count and lung ultrasonography were normal. On his 3rd day, the neonate presented asymptomatic sinus bradycardia associated with hypocalcemia. After three 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.

Discussion

The findings of this prospective study conducted in a single center over a six-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, which in turn influences the finding of SARS-CoV-2 positivity in the neonate.

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

results for SARS-CoV-2, similar to findings described in a recent meta-analysis[11] 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%) than reported in this previous meta-analysis.

Interestingly, RT-PCR positivity was detected for some AF and/or CB samples, with a more than four-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. To clarify this issue, it is necessary to cultivate the virus to determine viral replication in these samples.

In our sample, we observed SARS-CoV-2 RT-PCR positivity in 2.1% of colostrum samples. In one case, CB was also positive, though none of the other tested compartments was positive in the other 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,[25] and another recent study found one case (1/11) of SARS-CoV-2 positivity in maternal milk collected on the 5th day after delivery.[10]

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 (i) the need to reinforce personal protection for pregnant women mainly in the third trimester of pregnancy; (ii) whenever possible, delivery should be avoided during the acute phase of COVID-19 infection; and (iii) molecular tests (OPS or serum RT-PCR) 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.

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.

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

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 intrautero transmission.

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

In conclusion, our study confirms 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 in the third trimester and that whenever possible, delivery in the acute phase of the disease should be avoided.

References

1. Zhou F, Yu T, Du R, et al. Clinical course and risk-factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020; 395 (10229):1054–1062
2. Juan J, Gil MM, Rong Z, Zhang Y, Yang H, Poon LC. Effect of coronavirus disease 2019 (COVID-19) on maternal, perinatal and neonatal outcome: systematic review. *Ultrasound Obstet Gynecol*. 2020; 56 (1):15–27. doi: 10.1002/uog.22088.
3. Huntley BJF, Huntley ES, Di Mascio D, Chen T, Berghella V, Chauhan SP. Rates of maternal and perinatal mortality and vertical transmission in pregnancies complicated by severe acute respiratory syndrome coronavirus (SARS-COV-2) infection. A systematic review. *Obstet Gynecol*. 2020; 136(2):303–312. doi: 10.1097/AOG.0000000000004010.
4. Duran P, Berman S, Niermeyer S, et al. COVID-19 and newborn health: systematic review. *Rev Panam Salud Publica*. 2020; 27;44:e54. doi: 10.26633/RPSP.2020.54.
5. Yoon SH, Kang JM, Ahn JG. Clinical outcomes of 201 neonates born to mother with COVID-19: a systematic review. *Eur Rev Med Pharmacol Sci*. 2020; 24:7804–15. doi: 10.26355/eurrev_202007_22285.
6. Knight M, Bunch K, Vousden N, et al. Characteristics and outcomes of pregnant women 581 hospitalized with confirmed SARS-CoV-2 infection in the UK: a national cohort study using the 582 UK Obstetric Surveillance System (UKOSS). *BMJ*. 2020; 8;367:m2107. doi: 10.1136/bmj.m2107

7. Mullins E, Evans D, Viner RM, O´Brien P, Morris E. Coronavirus in pregnancy and delivery: rapid review. *Ultrasound Obstet Gynecol*. 2020; 55(5):586–92. doi:10.1002/uod.22014.
8. Yan J, Guo J, Fan C, et al. Coronavirus disease 2019 in pregnant women: a report based on 116 cases. *Am J Obstet Gynecol.* 2020; 223 (1):111.e1–111.e14.doi: 10.1016/j.ajog.2020.04.014.
9. Dashraath P, Wong JLJ, Lim MXK, et al. Coronavirus Disease 2019 (COVID-19) pandemic and pregnancy. *Am J Obstet Gynecol*. 2020; 222(6):521–31. doi: 10.1016/j.ajog.2020.03.021.
10. Fenizia C, Biasin M, Cetin I, Vergani P, Mileto D, Spinillo A. Analysis of SARS-CoV-2 vertical transmission during pregnancy. *Nat Commun*. 2020; 11(1):5128. doi: 10.1038/s41467-020-18933-4.
11. Kotlyar A, Grechukhina O, Chen A, et al. Vertical transmission of COVID-19: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2020; S0002-9378(20)30823-1. doi: 10.1016/j.ajog.2020.07.049.
12. Fan C, Lei D, Fang C, et al. Perinatal transmission of COVID-19 associated SARS-COV-2: should we worry? *Clin Infect Dis. 2020*; ciaa226. doi:10.1093/cid/ciaa226.
13. Chen H, Gui C, Luo F, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet.* 2020; 395(10226): 809–15. doi: 10.1016/S0140-6736(20)30360-3
14. Dong L, Tian J, He S, et al. Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. *JAMA*. 2020; 323(18):1846–48. doi: 10.1001/jama.2020.4621

15. Zeng L, Xia S, Yuan W, Yan K, Xiao F, Shao J. Neonatal early-onset infection with SARS-COV-2 in 33 neonates born to mothers with COVID-19 in Wuhan, China. *JAMA Pediatr*. 2020; 174(7):722–25. doi: 10.1001/jamapediatrics.2020.0878.
16. Yang Z, Liu Y. Vertical transmission of severe acute respiratory syndrome coronavirus 2: a systematic review. *Am J Perinatol*. 2020; 37(10):1055-60. doi: 10.1055/s-0040-1712161.
17. Egloff C, Vauloup-Fellous C, Picone O, Mandelbrot L, Roques P. Evidence and possible mechanisms of rare maternal-fetal transmission of SARS-CoV-2. *J Clin Virol.* 2020; 128:104447. doi: 10.1016/j.jcv.2020.104447.
18. Wang W, Xu Y, Gao R, Lu R, Han K, Lu G. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA.* 2020; 323(18):1843–44. doi: 10.1001/jama.2020.3786
19. Tony Zitek. The appropriate use of testing for COVID-19. *West J Emerg Med.* 2020; 13;21(3):470–72.doi: 10.5811/westjem.2020.4.47370.
20. Visseaux B, Le Hingrat Q, Collin G, et al. Evaluation of the RealStar® SARS-CoV-2 RT-PCR kit RUO performances and limit of detection. *J Clin Virol.* 2020; 129:104520. doi: 10.1016/j.jcv.2020.104520.
21. Costa SF, Buss L, Espinoza EPS, et al. Performance of a qualitative rapid chromatographic immunoassay to diagnose COVID-19 in patients in a middle-income country. *J Clin Virol.* 2020; 131: 104592. doi: 10.1016/j.jcv.2020.104592
22. Lennette EH, Schmidt NJ, editors. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th ed, Chapter 3. Washington, D.C. American Public Health Association,1979.
23. Ammerman NC, Beier-Sexton M, Azad AF. Growth and maintenance of Vero cell lines. Curr Protoc Microbiol. 2008; Appendix 4: Appendix 4E. doi: 10.1002/9780471729259.mca04es11.

24. Harcourt J, Tamin A, Lu X, et al. Isolation and characterization of SARS-CoV-2 from the first US COVID-19 patient. *bioRxiv* 2020. Preprint doi: 10.1101/2020.03.02.972935.

25. Chambers C, Krogstad P, Bertrand K, Contreras D, Tobin NH, Bode L, et al. Evaluation for SARS-Cov-2 in breast milk from 18 infected women. *JAMA.* 2020; 324(13):1347–48. doi:10.1001/jama.2020.15580

26. Voourdow B, Rockx B, Jaenisch T, et al. Performance of Zika Assays in the Context of Toxoplasma gondii, Parvovirus B19, Rubella Virus, and Cytomegalovirus (TORCH) Diagnostic Assays. *Clin Microbiol Rev*. 2019; 33(1):e00130–18. doi: 10.1128/CMR.00130-18.

27. Mark EG, Golden WC, Gilmore MM, et al. Community-Onset Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Young Infants: A Systematic Review. *J Pediatr*. 2020; S0022-3476(20)31133-1. doi:10.1016/j.jpeds.2020.09.008.

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*	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

*Other: cardiac disease, lung disease, hypothyroidism, anemia, neurological disorders. Severe COVID-19: required oxygen supply or ICU admission. ICU: intensive care unit; NPS: nasopharyngeal swab; SD: standard deviation; IQR: interquartile range

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).
462

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.
464

465

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

Case	Diag	COVID-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

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

Table 4. 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		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)	
Second	0 (0)	31(33.7)	0.007*
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†
Prepregnancy 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*

Data are presented as the number (percentage) and 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.

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†
Prepregnancy 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.

Table 6. 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		
	Positive SARS-CoV-2 RT-PCR (n= 8)	**Negative SARS-CoV-2 RT-PCR (n= 46)**	**p**
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*

Data are presented as the number (percentage) and median (range).
ICU: intensive care unit * Fisher exact test; † Mann-Whitney test.

Figure legend: Flow-chart of the study population. HU: Hospital Universitario, HC: Hospital das Clinicas.