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BRIEF REPORT

Vertical transmission of SARS-CoV-2 infection and preterm birth

Bianca Pulinx[1] · Davy Kieffer[1] · Ian Michiels[2] · Stéphanie Petermans[2] · David Strybol[3] · Sigrun Delvaux[3] · Marcella Baldewijns[4] · Marijke Raymaekers[5] · Reinoud Cartuyvels[5] · Wim Maurissen[1]

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Abstract
Viral infections are common complications of pregnancy, with a wide range of obstetric and neonatal sequelae. Currently, there are limited data on whether SARS-CoV-2 is vertically transmitted in pregnant women tested positive for the virus. Here we describe a case of a known SARS-CoV-2-positive woman giving preterm birth to two fetuses with SARS-CoV-2 positive testing in placental tissue and amniotic fluid. The placental histological examinations showed chronic intervillositis and extensive intervillous fibrin depositions with ischemic necrosis of the surrounding villi.

Keywords SARS-CoV-2 · Vertical transmission · Pregnancy

Introduction

In December 2019, a cluster of patients with pneumonia of unknown cause was first reported in Wuhan, China. A novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified as the culprit [1]. Infected patients might develop severe acute respiratory illness, with a relatively high likelihood of intensive care unit (ICU) admission and high mortality [2].

Viral infections are common complications of pregnancy, with a wide range of obstetric and neonatal sequelae. Some viruses cause mild maternal morbidity and generally have no impact on the pregnancy. Other viruses, such as cytomegalovirus (CMV), are typically asymptomatic or mild in the pregnant patient but can cause congenital infection with serious fetal and neonatal consequences [3].

Viruses can gain access to the decidua and placenta by ascending from the lower reproductive tract or via hematogenous transmission in which the virus circulating in the maternal bloodstream enters the placental villus, containing the fetal blood vessels, and is as such transmitted to the fetus [4].

Currently, there are limited data on whether the virus is vertically transmitted in pregnant women tested positive for SARS-CoV-2 [5–12].

Here we describe a case of known SARS-CoV-2-positive woman pregnant with twins giving preterm birth to two fetuses with SARS-CoV-2 positive testing in placental tissue and amniotic fluid.

Case report

A 30-year-old woman (gravida 2, para 1) presented with rhinitis and fever (39.2 °C) to our emergency department (ED) at 22 weeks of gestation. The patient was pregnant with dichorionic diamniotic (DCDA) twin and was recently diagnosed with gestational diabetes mellitus. She tested negative for toxoplasma IgG and IgM antibodies at 21 weeks of gestation. The patient tested positive (Ct 23) for SARS-CoV-2 RT-PCR on a nasopharyngeal swab. Blood pressure and heart rate were normal, oxygen saturation was 98% while breathing ambient air and C-reactive protein was slightly elevated

The manuscript has not been previously published nor is not being considered for publication elsewhere.

✉ Wim Maurissen
wim.maurissen@stzh.be

[1] Department of Clinical Biology, Sint-Trudo Hospital, Diestersteenweg 100, 3800 Sint-Truiden, Belgium

[2] Department of Gynaecology, Sint-Trudo Hospital, Sint-Truiden, Belgium

[3] Department of Pathology, Sint-Trudo Hospital, Sint-Truiden, Belgium

[4] Department of Pathology, University Hospitals Leuven, Leuven, Belgium

[5] Department of Clinical Biology, Jessa Hospital, Hasselt, Belgium

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(Table 1). Her chest X-ray showed no abnormalities. The patient was discharged from the hospital on the same day.

At 24 weeks of gestation, the patient presented at the ED with low abdominal pain and back pain. At this moment, the COVID-19 symptoms had disappeared. The fetal demise of fetus one was diagnosed and fetus two showed fetal heart rate decelerations. Due to unstoppable labor, the patient gave preterm birth to two fetuses with prepartal intrauterine death of fetus one and prepartal death of fetus two. Since no other cause for fetal demise was identified, extensive viral diagnostic testing was initiated, including COVID-19.

Both placental tissue samples (Ct 33 and Ct 30) and the amniotic fluid (Ct 23) tested positive for SARS-CoV-2 RT-PCR, as well as the maternal blood sample at the time of birth (Ct 35). Samples of both amniotic sac tested negative for SARS-CoV-2. The patient showed immunity for CMV and Rubella and tested negative for the other TORCH infections (which include toxoplasmosis, syphilis, and herpes). Furthermore, urine culture was negative as well as the *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (CT-NG) PCR (Table 1).

Both placentas showed extensive intervillous fibrin depositions and ischemic necrosis of the surrounding villi. Aggregates of histiocytes and cytotoxic T lymphocytes in the intervillous space were also present and confirmed with immunohistochemical stainings for CD68, CD3, and CD8 (Figs. 1 and 2). These findings supported the diagnosis of chronic intervillositis. In the fetal circulation, there was nuclear debris and an increase in erythroblasts, as can be seen in fetal hypoxia. There was no evidence of chorioamnionitis. Furthermore, viral localization in the placental syncytiotrophoblast cells was confirmed by immunohistochemistry with the Genetex SARS-CoV-2 antibody (Figs. 3 and 4).

Discussion

We report the first case of SARS-CoV-2 detection in both amniotic fluid and placental tissue from preterm fetuses born to a SARS-CoV-2-positive mother. The placental histological examinations showed chronic intervillositis and extensive intervillous fibrin depositions with ischemic necrosis of the surrounding villi. These findings support the possibility of vertical transmission of SARS-CoV-2 infection, and miscarriage due to the infection cannot be ruled out.

In support of our data, there is a reported case of miscarriage during the second trimester of the pregnancy in a SARS-CoV-2-positive mother, with placental SARS-CoV-2 infection, as well as histological findings in the placenta

Table 1 Laboratory results of SARS-CoV-2-positive mother

	Reference range	21-week gestation	22-week gestation	24-week gestation
Hemoglobin (g/dL)	12.1–16.1		12.0	13.1
White cell count ($\times 10^3/\mu L$)	4.0–10		6.9	10
Neutrophil count ($\times 10^3/\mu L$)	1.5–7.5		5.9	6.3
Lymphocyte count ($\times 10^3/\mu L$)	1.0–3.5		0.3	3.1
Platelet count ($\times 10^9/L$)	150–400		204	360
C-reactive protein (mg/L)	< 5		17.9	6.6
Alanine aminotransferase (U/L)	< 31		9	14
Aspartate aminotransferase (U/L)	< 32		15	17
γ-Glutamyltransferase (U/L)	5–36		8	12
Sodium (mmol/L)	136–145		131	
Potassium (mmol/L)	3.4–4.5		3.8	
Chloride (mmol/L)	98–106		95	
CMV IgM (ratio)	< 0.70	0.22 (negative)		
CMV IgG (IU/mL)	< 0.5	368.4 (immune)		
Toxoplasma IgG (IU/mL)	< 1.00	< 0.13 (negative)		< 0.13 (negative)
Toxoplasma IgM (ratio)	< 0.80	0.22 (negative)		0.95 (borderline)*
Rubella IgG (IU/mL)	< 10			119.4 (immune)
Rubella IgM				Negative
Syphilis antibodies (ratio)	< 1.0			< 0.2 (negative)
Herpes Simplex IgM				Negative
CT-NG PCR urine				Negative

*Confirmation negative

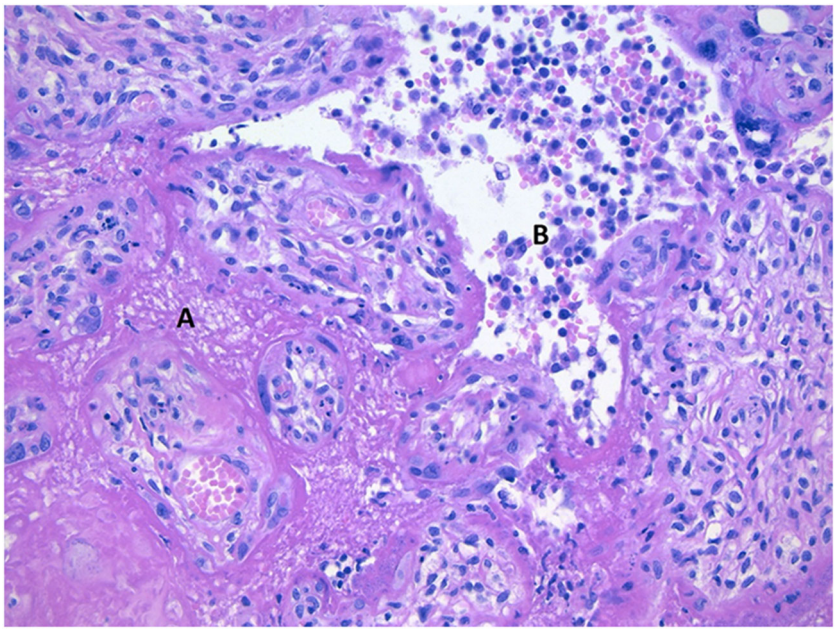

Fig. 1 High power view (× 20) of the placenta with intervillous fibrin depositions (A) and aggregates of histiocytes and cytotoxic T lymphocytes (B)

demonstrating inflammation. However, amniotic fluid and vaginal swabs collected during delivery tested negative for SARS-CoV-2, as well as the fetal swabs [11].

Other studies focused on COVID-19 presentation in the third trimester of pregnancy. Recently, a severe presentation of COVID-19 in pregnancy, requiring mechanical ventilation, was reported. Sixteen hours after caesarian delivery, the neonatal nasopharyngeal swab was SARS-CoV-2 RT-PCR positive. The neonate was isolated immediately after birth, without delayed cord clamping or skin-to-skin contact. This early reported positive PCR in the neonate suggests that vertical transmission is possible [8]. Furthermore, Dong and colleagues reported a newborn with elevated IgG and IgM antibodies to SARS-CoV-2 born to a SARS-CoV-2-positive mother. The elevated IgM antibodies were detected in a blood sample drawn 2 h after birth. The production of IgM and IgG antibodies typically occurs several days after exposure, with IgM antibodies appearing first. The presence of these antibodies indicates that the newborn had been exposed to the SARS-CoV-2 virus supporting the possibility of vertical transmission [10]. Our data are in alignment with Penfield et al., who detected the presence of SARS-COV-2 in placental and membranes samples by RT-PCR, in women with severe to critical COVID-19 at the time of delivery [12].

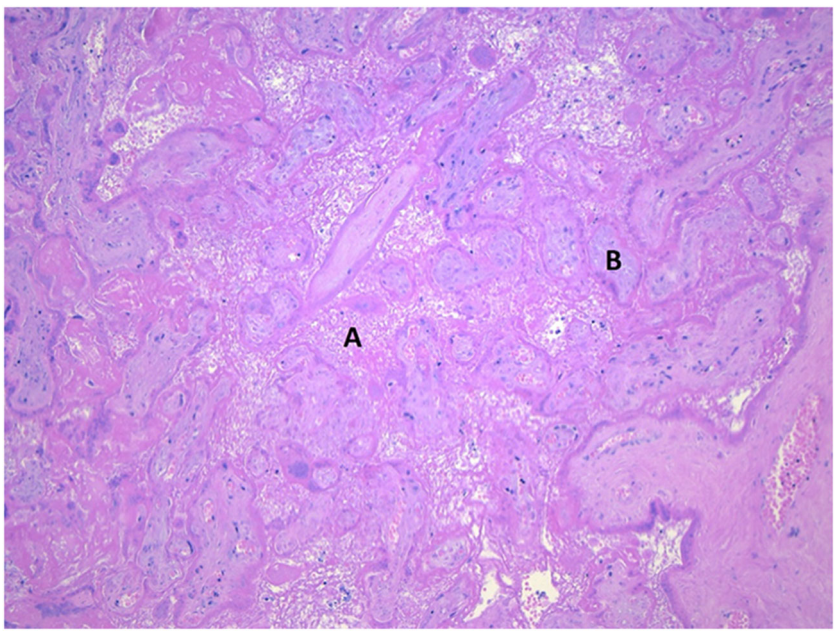

Fig. 2 Lower power view (× 10) of the placenta with intervillous fibrin depositions (A) and ischemic necrosis of the surrounding villi (B)

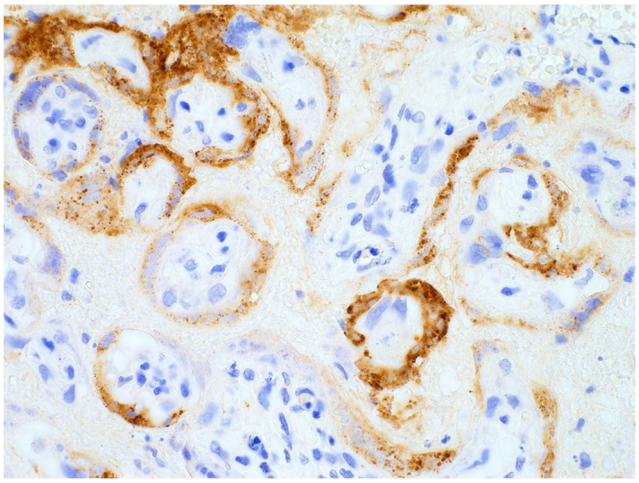

Fig. 3 High power view (x40) showing viral localization in the placental syncytiotrophoblast cells

Recently, Shwartz reported that there were no cases of either severe pneumonia or maternal deaths in 38 pregnant COVID-19-positive women. Furthermore, the neonates delivered of these women were all confirmed RT-PCR SARS-CoV-2 negative, as were the placentas [9]. Chen and colleagues investigated the possibility of intrauterine transmission of COVID-19 infection by testing amniotic fluid, cord blood, and neonatal throat swabs at birth. All collected samples were negative for SARS-CoV-2. Both studies suggest that intrauterine transmission of COVID-19 is unlikely [5].

Some limitations should also be addressed. First, we did not evaluate the presence of the virus in samples or tissue of the fetuses and no bacterial culture of the amniotic fluid was performed. Second, this report is limited to a single case. Third, we cannot rule out other causes of miscarriage, such as other viral or bacterial infections. Further investigation is necessary to ascertain potential intrauterine vertical transmission in women with COVID19 and possible fetal and neonatal consequences.

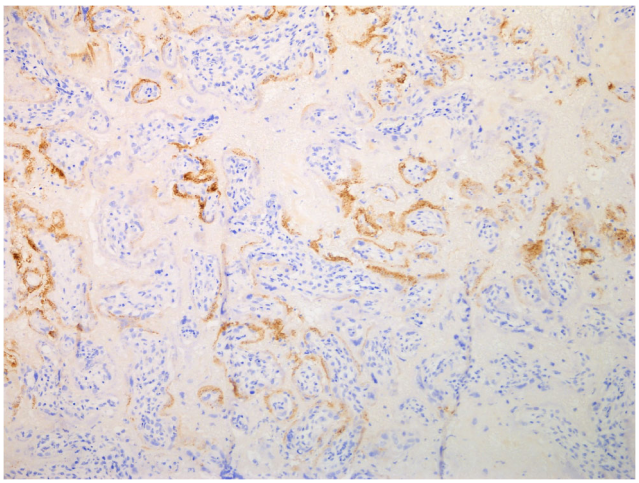

Fig. 4 Lower power view (x10) showing viral localization in the placental syncytiotrophoblast cells

Methods

Sample collection

At 21, 22, and 24 weeks of gestation, maternal venous blood was collected. The maternal nasopharyngeal swab was collected at 22 weeks of gestation and preserved in a 3-mL viral transport medium (Copan UTM, Brescia, Italy). A maternal urine sample was obtained on the day of delivery (24-week gestation). An amniotic fluid swab was collected immediately post-partum in isolation operating room and preserved in a 3-mL viral transport medium (Copan UTM, Brescia, Italy). Both placentas and both amniotic sacs were collected for histopathological examination.

Real-time reverse transcription PCR

Ten sections of each formalin-fixed paraffin-embedded tissue were treated with Ultraclear® (VWR International, Radnor, PA) and ethanol (Emprove Expert Ethanol 96%, Merck Millipore, Burlington, MA) to remove paraffin.

For all samples (pretreated tissue, nasopharyngeal swab EDTA blood, amniotic fluid), a lysis step was performed by adding Maxwell Lysis Buffer (Promega, Madison, WI), ethanol, and proteinase K (Qiagen, Hilden, Germany). An extraction and amplification control (Phocine Distemper Virus, kindly provided by the Department of Viroscience, Erasmus Medical Centre Rotterdam) was added to each sample. Nucleic acid extraction was done with the Maxwell RSC Viral TNA kit (Promega, Madison, WI) on the Maxwell RSC Instrument (Promega, Madison, WI) according to the manufacturers' instructions. Our in-house rRT-PCR is based on the CDC oligonucleotide primers and probes for the detection of the viral nucleocapsid (N) gene of 2019-nCoV (2019-nCoV_N1) and was performed on the Quantstudio 7 flex (ThermoFisher, Waltham, MA) (https://www.fda.gov/media/134922/download). Each tissue sample was analyzed undiluted and in a 1:10 dilution.

Histopathological examination

Hematoxylin and eosin-stained slides were examined for morphological analysis performed by three experienced pathologists (D.S., S.D., and M.B.). Immunostains were performed by using a standard immunohistochemical protocol.

Clinical laboratory parameters

Routine biochemical and serological laboratory parameters were analyzed on the Cobas 6000 (Roche Diagnostics, Basel, Switzerland). Hematological parameters were determined on the XN-3100 (Sysmex Corporation, Kobe, Japan).

CT-NG PCR was performed on the GeneXpert (Cepheid, Sunnyvale, CA).

Author's contributions All authors have seen and approved the manuscript, contributed significantly to the work.

Availability of data and material (data transparency) Yes

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Patient gave consent for this case to be published.

References

1. Zhu N, Zhang D, Wang W et al (2020) A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382(8): 727–733
2. Huang C, Wang Y, Li X et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395(10223):497–506
3. Rogan SC, Beigi RH (2019) Treatment of viral infections during pregnancy. J Perinatol 46(2):235–256
4. Racicot K, Mor G (2017) Risks associated with viral infections during pregnancy. J Clin Invest 127(5):1591–1599
5. Chen H, Guo J, Wang C et al (2020) Clinical characteristics and interuterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 395:809–815
6. Della Gatta AN, Rizzo R, Pilu G, Simonazzi G (2020) COVID19 during pregnancy: a systematic review of reported cases. Am J Obstet Gynecol. https://doi.org/10.1016/j.ajog.2020.04.013
7. Yang H, Sun G, Tang F et al (2020) Clinical features and outcomes of pregnant women suspected of coronavirus disease 2019. J Infect. https://doi.org/10.1016/j.jinf.2020.04.003
8. Alzamora MC, Paredes T, Caceres D, Webb CM, Valdez LM, La Rosa M (2020) Severe COVID-19 during pregnancy and possible vertical transmission. Am J Perinatol. https://doi.org/10.1055/s-0040-1710050
9. Shwartz DA (2020) An analysis of 38 pregnant women with COVID-19, their newborn infants, and maternal-fetal transmission of SARS-CoV-2: maternal coronavirus infections and pregnancy outcomes. Arch Pathol Lab Med. https://doi.org/10.5858/arpa.2020-0901-SA
10. Dong L, Tian J, He S et al (2020) Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. JAMA. https://doi.org/10.1001/jama.2020.4621
11. Baud D, Greub G, Favre G et al (2020) Second-trimester miscarriage in a pregnant woman with SARS-CoV-2 infection. JAMA. https://doi.org/10.1001/jama.2020.7233
12. Penfield CA, Brubaker SG, Limaye MA et al (2020) Detection of SARS-CoV-2 in placental and fetal membrane samples. Am J Obstet Gynecol MFM. https://doi.org/10.1016/j.ajogmf.2020.100133