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Evidence of vertical transmission of SARS-CoV-2 and interstitial pneumonia in second-trimester twin stillbirth in asymptomatic woman. Case report and review of the literature

Luisa Patanè, MD; Massimiliano Cadamuro, PhD; Gabriella Massazza, MD; Serena Pirola, MD; Valentina Stagnati, MD; Chiara Comerio, MD; Marco Carnelli, MD; Marco Arosio, MD; Anna Paola` Callegaro, MD; Paola Tebaldi, MD; Elena Rigoli, MD; Andrea Gianatti, MD; Denise Morotti, BS

Data on the vertical transmission rate of COVID-19 in pregnancy are limited, although data reporting mother-fetal transmission in the second trimester of pregnancy are controversial.

We described a case of second-trimester twin stillbirth in a woman with SARS-CoV-2 infection in which placental and fetal markers of infection were detected, despite the absence of respiratory syndrome. The patient developed clinical chorioamnionitis and spontaneously delivered 2 stillborn infants. Placental histology and immunohistochemistry demonstrated SARS-CoV-2 infection mostly within the syncytiotrophoblast, and fetal autopsy showed the development of interstitial pneumonia.

Our findings demonstrated that in utero vertical transmission is possible in asymptomatic pregnant women with SARS-CoV-2 infection and that infection can lead to severe morbidity in the second trimester of pregnancy.

Introduction

There are few published reports regarding the possibility of severe SARS-CoV-2 vertical transmission during the first or second trimester of pregnancy.

This report described a case of SARS-CoV-2 vertical transmission in the second trimester of pregnancy of an asymptomatic woman with fetal SARS-CoV-2–related pneumonia leading to adverse fetal outcomes.

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From the Department of Obstetrics and Gynecology, ASST Papa Giovanni XXIII, Bergamo, Italy; Department of Molecular Medicine, University of Padua, Padua, Italy; Department of Pathology, ASST Papa Giovanni XXIII, Bergamo, Italy; Microbiology and Virology Unit, ASST Papa Giovanni XXIII, Bergamo, Italy; Biobank Unit, ASST Papa Giovanni XXIII, Bergamo, Italy.

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This study had ethical approval from the hospital institutional review board (approval number: 74-20) and signed-consent from the patients.

The authors report no conflict of interest.

Corresponding author: Luisa Patanè, MD. lpatane@asst-pg23.it

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EDITOR'S CHOICE

Case

A 35-year-old woman with a twin pregnancy with a positive nasopharyngeal swab polymerase chain reaction (PCR) test for SARS-CoV-2 was admitted at 20 weeks of gestation for preterm premature rupture of membranes (PPROM). The pregnancy was achieved through in vitro fertilization, and the patient received cervical cerclage at 16 weeks of gestation for cervical incompetence.

At admission, the patient was asymptomatic, with negative chest x-ray and normal laboratory tests. The obstetrical ultrasonography demonstrated a twin pregnancy, with normal growth of both fetuses and oligohydramnios of the first twin. The patient firmly refused cerclage removal, and following a negative vaginal swab test, a prophylactic antibiotic therapy was started.

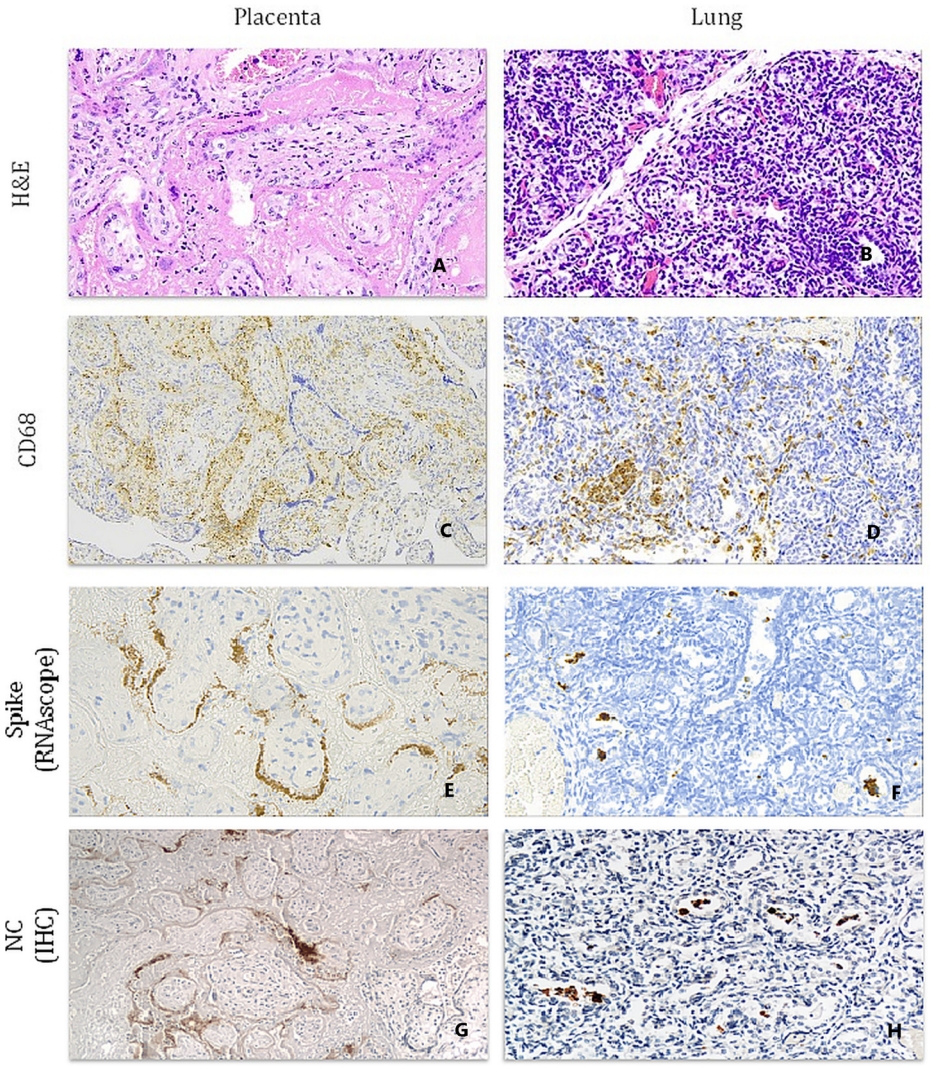
At 21.4 weeks of gestation, the patient's clinical condition deteriorated; she became febrile with increased leukocyte count and PCR values. Due to the onset of premature labor, cervical cerclage was removed, and the patient delivered 2 stillborn fetuses vaginally. The postpartum course showed an immediate improvement of maternal conditions, and the patient was discharged 3 days after delivery with normal laboratory tests.

Results

The placentas of both fetuses were collected, sampled, and analyzed at our pathology department by skilled pathologists (E.R. and P.T.).

At histologic analysis, both placentas showed lymphocytic and histiocytic inflammatory infiltrates and were

FIGURE
Histology, immunohistochemistry, in-situ hybridation on placenta and fetal lungs



Representative pictures of the placenta showing chronic histiocytic intervillositis with accumulation of mononuclear inflammatory cells in the intervillous space (**A** and **C**). The fetal lungs in the canalicular stage showed features of interstitial pneumonia with presence of neutrophilic infiltrate and cell debris in the alveolar ducts with increased histiocytic infiltrate (**B** and **D**). The placenta showing in situ hybridization (RNAscope Technology) for SARS-CoV-2 spike protein viral RNA (*brown dots*), resulting in positivity within the syncytiotrophoblast of multiple chorionic villi (**E**). IHC expression of SARS-CoV-2 NC protein in the chorionic villi resulting in a pattern of circumferential villous staining (**G**). Tissues from the lungs at the canalicular stage of the first fetus showed an expression of both SARS-CoV-2 spike protein viral RNA (*brown dots*, RNAscope) and SARS-CoV-2 NC (IHC) (**F** and **H**). Original magnification: all × 40.

IHC, immunohistochemical; *NC*, nucleocapsid.

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characterized by chronic intervillositis, with several macrophages in the intervillous spaces (Figure, A). Immunohistochemistry (IHC) confirmed chronic intervillositis showing the recruitment of CD68-positive macrophages (Figure, C). Moreover, the placentas showed diffuse perivillous fibrin deposition and trophoblast necrosis.

Fetal autopsies did not show any malformation, and at microscopic examination, the heart, liver, kidney, and thymus showed no sign of pathology. At the canalicular stage, Histological examination of the lung of the first fetus only, at canalicular stage, showed interstitial pneumonia features characterized by vascular congestion, neutrophilic infiltrate

TABLE 1
SARS-CoV-2 test results

Variable	SARS-CoV-2 RT-PCR frozen specimens	SARS-CoV-2RT-PCRFFPE specimens	IHC ab-NC SARS-CoV-2	ISH spike protein SARS-CoV-2	IHC ab-ACE2 receptor
First fetus					
Placenta	POS	POS	POS	POS	POS
Membranes	NA	POS	NA	NEG	NA
Cord	NA	POS	NEG	NEG	POS
Lung	NA	POS	POS	POS	NEG
Heart	NA	POS	NEG	NEG	NEG
Kidney	NA	POS	NEG	NEG	POS
Liver	NA	POS	NEG	NEG	NEG
Second fetus					
Placenta	POS	POS	POS	POS	POS
Membranes	NA	POS	NA	NEG	NA
Cord	NA	POS	NEG	NEG	POS
Lung	NA	POS	NEG	NEG	NEG
Heart	NA	POS	NEG	NEG	NEG
Kidney	NA	NEG	NEG	NEG	POS
Liver	NA	POS	NEG	NEG	NEG

FFPE, formalin-fixed paraffin embedded; *IHC ab-ACE2*, immunohistochemistry with antibody vs angiotensin-converting enzyme 2 receptor; *IHC ab-NC*, immunohistochemistry with antibody vs viral nucleocapsid protein; *ISH* spike protein, in situ hybridization with an RNA probe for the viral spike protein; *NA*, not available; *NEG*, negative; *POS*, positive; *RT-PCR*, reverse transcription-polymerase chain reaction.

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and cell debris in the alveolar ducts with slight increase in histiocytic infiltrate (Figure, B–D).

Formalin-fixed paraffin-embedded and frozen specimens collected at the fetal and maternal sides of the placenta were analyzed using reverse transcription real time-PCR (RT-PCR), IHC, and in situ hybridization (RNAscope Technology), to detect the presence and localization of SARS-CoV-2. Using RT-PCR, SARS-CoV-2 was detected in all tissue samples (fetal lung, kidney, heart, and liver and maternal placenta, cords, and membranes), except for the kidney of the second fetus. Both IHC and in situ hybridization confirmed the positivity of the placentas and lungs of the first fetus (Table 1 and Figure, E–H).

Discussion

Literature outlined that mother-fetus vertical transmission of COVID-19 is a rare event affecting approximately 2% of maternal infections[1]; this event is more common during the third trimester of pregnancy, but it could be found in all trimesters of pregnancy.[2] Vertical transmission is usually associated with COVID-19–related maternal symptoms, such as fever, pulmonary infection, and multiorgan involvement, with an increase of premature labor and preeclampsia rates.[3]

Regarding placental pathology in SARS-CoV-2 infection, it is already known that the syncytiotrophoblast is putatively the main structure most susceptible to infection cell types. The typical pathologic findings detectable at the placental level and associated with SARS-CoV-2 infection are chronic histiocytic intervillositis and trophoblast necrosis, in both viable and stillbirth-complicated pregnancies, such as in the recent case. When the syncytiotrophoblast is not infected, vertical transmission is unlikely, and the placenta could seem histologically normal or show elements of maternal or fetal malperfusion.[4,5] According to previously published classifications, this case could be considered as a confirmed case of vertical transmission because of the positivity of both placental and fetal tissues.[6]

To date, only few cases of possible vertical transmission in the first and second trimesters of pregnancy have been published.

Baud et al[7] reported a case of second-trimester miscarriage in a symptomatic mother with SARS-CoV-2 infection. Placental histology demonstrated typical inflammatory lesions suggestive of SARS-CoV-2 infection, but amniotic fluid and vaginal and fetal swabs tested negative. Hosier et al[8] described a case of SARS-CoV-2 placental infection in the second trimester of pregnancy complicated by severe preeclampsia and COVID-19–related pulmonary disease. The patient opted for pregnancy termination, and the analysis of the placenta showed a positive SARS-CoV-2 result in the syncytiotrophoblast with a dense macrophage infiltration. RT-PCR analysis on the placenta and umbilical cord was positive for SARS-

CoV-2, but fetal tissue analysis could not demonstrate evidence of fetal infection.

Using RT-PCR analysis, Pulinx et al[9] reported the positivity of amniotic fluid and placental tissues to SARS-CoV-2 from 2 fetuses born that immediately died at 24 weeks of gestation after the mother contracted the virus with the onset of symptoms at 22 weeks of gestation. At the time of delivery, the patient was asymptomatic. The placental histology showed chronic intervillositis with ischemic villous necrosis, and viral localization in the syncytiotrophoblast was confirmed by IHC; however, the authors were unable to confirm fetal infection because of the inability to analyze fetal tissues.

Valdespino et al[10] reported a rare case of spontaneous twin abortion at the first trimester of pregnancy in a symptomatic mother with COVID-19. RT-PCR of the placenta, kidneys, and lungs for SARS-CoV-2 of 1 fetus returned positive, confirmed by immunofluorescence and electron microscopy analysis. Moreover, histology of the placenta showed infarctions, perivillous fibrin deposition, and chronic intervillositis with CD163+ inflammatory cells. The fetal lung was at the pseudoglandular stage and was characterized by CD8+ macrophages infiltration without any sign of overt pneumonia.

In our report, we demonstrated SARS-CoV-2 infection using 2 different techniques, in situ hybridization (RNAscope) and IHC, to detect viral RNA and viral protein, respectively, in placental and fetal samples to reduce false negatives because of technical issues. RT-PCR has been proven to be the most sensitive technique in detecting COVID-19 in all tissues tested except for the kidney of the second fetus. Both IHC and in situ hybridization demonstrated that SARS-CoV-2 reached the lungs of the first fetus through a hematogenous mechanism, despite both techniques being less sensitive than RT-PCR. In adult life, the virus enters the lung epithelia and binds to the ACE2 receptor; however, it is known that this pathway cannot be used by the virus in fetal life because the expression of the ACE2 receptor in the lung is known to be absent throughout pregnancy.[11]

Moreover, we described the typical histologic features of fetal pneumonia in association with the presence of the virus in the fetus. Similar to what is reported in adult patients, fetal pneumonia is characterized by vascular congestion, accumulation of neutrophilic infiltrate, and presence of cell debris in the alveolar ducts accompanied by a small increase in histiocytic infiltrates.[12]

In our case, the pregnant woman was asymptomatic but manifested serious adverse pregnancy outcomes, and the placentas showed typical features already associated with SARS-CoV-2 vertical transmission.[13] The obstetrical complications (PPROM and cervical insufficiency) could have allowed SARS-CoV-2 entrance through the placenta to infect the fetal tissues, or SARS-CoV-2 infection together with inflammatory conditions could be the primum movens of the superimposed clinical chorioamnionitis. All data relating to the studies analyzed are summarized in Table 2.

TABLE 2
Vertical transmission in the first and second trimester of pregnancy, data from published studies

Study	GA (wk)	COVID-19–related maternal symptoms	Placental histologic findings	SARS-CoV-2 detection methods and findings	
Evidence of SARS-CoV-2 in both the fetus and placenta				Fetus	Placenta
Our case	21.4 Twin	Asymptomatic	Chronic intervillositis, maternal vascular malperfusionfeatures, chorioamnionitis,funisitis	Lung (positive) Kidney (positive) Heart (positive) Liver (positive)	Placenta (positive) Cord (positive) Membranes (positive)
Valdespino-Vázquez et al[10]	13.0 Twin	Fever, myalgia,headache, pharyngodynia	Placental infarctions, diffuse perivillous fibrin, active chronic intervillositis	Lung (positive) Kidney (positive)	Placentas (positive)
Evidence of SARS-CoV-2 only in placental tissue				Fetus	Placenta
Baud et al[7]	19.0 Singleton	Fever, myalgia,diarrhea, cough	Inflammatory infiltrates composed of neutrophils and monocytes in the subchorialspace and unspecific increased intervillous fibrin deposition,funisitis	Lung (negative) Liver (negative) Thymus (negative) Anus (negative)	Placenta (positive) Membranes (positive)
Hosier et al[8]	22.0 Singleton	Fever, myalgia,diarrhea, nausea, cough	Perivillous fibrin, histiocytic intervillositis	Lung (negative) Heart (negative)	Placenta (positive) Cord (positive)
Pulinx et al[9]	24.0 Twin	Fever and rhinitis at 22 wk	Chronic intervillositis	Not available	Placentas (positive) Amniotic fluid (positive) Membranes (negative)

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This case report demonstrated that SARS-CoV-2 infection in asymptomatic women could be related to severe adverse fetal and maternal outcomes in the second trimester of pregnancy. These findings should be considered when counseling pregnant women with SARS-CoV-2 infection to guarantee optimal care. ■

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ajogmf.2022.100589.

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