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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.

L Patanè[1], M Cadamuro[2], G Massazza[3], S Pirola[1], V Stagnati[1], C Comerio[1], M Carnelli [1], M Arosio[4,5], AP Callegaro [4,5], P Tebaldi[3], E Rigoli [3], A Gianatti [3], D Morotti[3].

[1] Department of Obstetrics and Gynecology ASST - Papa Giovanni XXIII - Bergamo

[2] Department of molecular medicine, University of Padova

[3] Department of Pathology ASST - Papa Giovanni XXIII - Bergamo

[4] Microbiology and Virology Unit - ASST - Papa Giovanni XXIII - Bergamo

[5] Biobank Unit ASST - Papa Giovanni XXIII - Bergamo

We had ethical approval of our Hospital institutional review board (approval number 74 -20), as well as signed consent from the patient.

No conflict of interest is present within our research.

CORRESPONDING AUTHOR

Luisa Patanè

Department of Obstetrics and Gynecology ASST - Papa Giovanni XXIII

Bergamo

Italy

lpatane@asst-pg23.it

ABSTRACT

Data on the vertical transmission rate of COVID-19 in pregnancy are limited, while 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 positive for SARS-CoV-2 in which, despite the absence of respiratory syndrome, placental and fetal markers of infection were detected. The patient developed a clinical chorioamnionitis and spontaneously delivered two stillborn infants. Placental histology and immunohistochemistry demonstrated SARS-CoV-2 infection mostly within the syncytiotrophoblast and the fetal autopsy showed development of interstitial pneumonia.

Our findings demonstrate that, in utero vertical transmission is possible, also in asymptomatic SARS-CoV-2 pregnant women 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 acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vertical transmission during first or second trimester of pregnancy.

This report describes a case of SARS-CoV-2 vertical transmission in a second trimester asymptomatic woman, with fetal SARS-CoV-2-related pneumonia leading to adverse fetal outcome .

Case

A 35 years old twin pregnant, SARS-CoV-2 PCR positive at nasopharyngeal swab , was admitted at 20 weeks for preterm premature rupture of membranes. The pregnancy was obtained by *in vitro* fertilization (IVF) and the patient received cervical cerclage at 16 weeks for cervical incompetence.

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

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

RESULTS

Placentas of both fetuses were collected, sampled and analyzed at our Pathology Department by a skilled pathologist (ER/PT).

At histological analysis, both placentas showed lymphocytic and histiocytic inflammatory infiltrate and were characterized by chronic intervillositis, with numerous macrophages in the intervillous spaces (figure 1.A). Immunohistochemistry (IHC) confirmed chronic intervillositis showing the recruitment of CD68-positive macrophages (figure 1.C). Moreover, the placentas showed diffuse peri-villous fibrin deposition and trophoblast necrosis.

Fetal autopsies did not show any malformations and, at microscopical examination, heart, liver, kidney and thymus showed no signs of pathologies. Histological examination of the lung of the first fetus only, at canalicular stage, showed interstitial pneumonia features

characterized by vascular congestion, neutrophilic infiltrate and cell debris in the alveolar ducts with slight increase in histiocytic infiltrate (figure 1.B-D).

Formalin-fixed paraffin-embedded (FFPE) and frozen specimens collected by fetal and maternal side were then analyzed by real time-PCR (RT-PCR), IHC, and in situ hybridization (RNAscope™ technology), in order to detect the presence and localization of SARS-CoV-2. By 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 immunohistochemistry and in situ hybridization confirmed positivity of the placentas and the lungs of the first fetus (Table 1 and figure 1-E-F-G-H).

DISCUSSION

Literature outlined that mother-fetus vertical transmission of coronavirus disease 2019 (COVID-19) is a rare event affecting approximately 2% of maternal infection (1); this event is more common during the third trimester of pregnancy, but it could be found in all t trimesters (2). Vertical transmission is usually associated with COVID-19-related maternal symptoms such as fever, pulmonary infection and multi-organ involvement, with an increase of premature labor and pre-eclampsia rates (3).

Regarding placental pathology in SARS-CoV2 infection, it is already known that syncytiotrophoblast is putatively the main and most susceptible to infection cell type present at the syncytiotrophoblast. The typical pathological findings detectable at placental level and associated with SARS-CoV-2 infection are chronic histiocytic intervillositis and trophoblast necrosis, in both viable and stillbirth complicated pregnancies, as in the present case. When syncytiotrophobalst in not infected, vertical transmission is unlikely and the placenta could appear histologically normal or show elements of maternal or fetal malperfusion (4,5). According to previous published classification, this case could be considered as a confirmed case of vertical transmission due to the positivity of both placental and fetal tissues (6).

To date, only few cases of possible vertical transmission in first and second trimester have been already published.

Baud and colleagues (7) reported a case of second trimester miscarriage in a SARS-CoV2 symptomatic mother. Placental histology demonstrated typical inflammatory lesions suggestive of SARS-CoV-2 infection, but amniotic fluid, vaginal and fetal swabs tested negative. Hosier et al. (8) described a case of SARS-CoV-2 placental infection in second trimester pregnancy complicated by severe pre-eclampsia and COVID-19-related

pulmonary disease. The patient opted for pregnancy termination and analysis of the placenta showed SARS-CoV-2 positivity in the syncytiotrophoblast with a dense macrophage infiltration. RT-PCR analysis on placenta and umbilical cord were positive for SARS-CoV-2, but fetal tissue analysis could not demonstrated evidences of fetal infection.

Pulinx and colleagues (9) reported the positivity to SARS-CoV-2, at RT-PCR analysis, of amniotic fluid and placental tissues from two fetuses born and immediately died at 24 weeks after that the mother contract the virus with symptoms at 22 weeks. At the time of delivery she was asymptomatic. The placental histology showed chronic intervillositis with ischemic villous necrosis and viral localization in syncytiotrophobalst was confirmed by immunohistochemistry, but the authors were unable to confirm fetal infection due to the inability to analyze fetal tissues.

Valdespino and colleagues (10) reported a rare case of spontaneous twin abortion at first trimester of pregnancy in a mother with symptomatic COVID-19. RT-PCR for SARS-CoV-2 ~~resulted~~ was positive in placenta, kidneys and lungs of one fetus, and confirmed by immunofluorescence and electron microscopy analysis. Moreover, histology of the placenta showed infarctions, perivillous fibrin deposition and chronic intervillositis with CD163+ inflammatory cells. Fetal lung was at pseudoglandular stage, and was characterized by CD8+ macrophages infiltration without signs of overt pneumonia.

In ~~this~~ our report we demonstrated SARS-CoV-2 infection using two different technique, in situ hybridization (RNAscope) and IHC to detect viral RNA and viral protein, respectively, in placental and fetal samples in order to reduce false negatives due to technical issues. RT-PCR has been proven to be the most sensitive technique allowing the detection of COVID-19 in all tissue tested except for the kidney of the second fetus. Both IHC and in situ hybridization, despite being less sensitive respect to RT-PCR, demonstrated that SARS-CoV-2 reached the lungs of the first fetus through hematogenous mechanism. In adult life, the virus enters the lungs epithelia binding to ACE-2 receptor but is known that this pathway cannot be used by virus in fetal life, because the expression of ACE-2 receptor in lung is known to be absent throughout pregnancy (11).

Moreover, we described the typical histological features of fetal pneumonia in association to the presence of the virus in the fetus. Similarly, to what reported in adult patients, fetal pneumonia is characterised by vascular congestion, accumulation of neutrophilic infiltrate and presence of cell debris in the alveolar ducts accompanied by a small increase in histiocytic infiltrate (12).

In our case, the pregnant woman was asymptomatic but manifested serious adverse pregnancy outcome and placentas showed typical features already associated with SARS-CoV-2 vertical transmission (13). The obstetric complications (premature rupture of membranes and cervical insufficiency) could have allowed SARS-CoV-2 entrance trough placenta to infect fetal tissue or, alternatively, 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.

This case report demonstrates that SARS-CoV-2 infection, in asymptomatic women, could be related with severe adverse fetal and maternal outcomes also in second trimester of pregnancy. These findings should to be taken into account when counselling pregnant women with SARS- CoV-2 infection in order to guarantee the optimal care.

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Figure1. Histological, immunohistochemical and in situ hybridization findings on placenta and fetal lungs.

Representative pictures of placenta showing chronic histiocytic intervillositis with accumulation of mononuclear inflammatory cells in the intervillous space (1.A,C). Notably, fetal lungs in canalicular stage showed features of interstitial pneumonia with presence of neutrophilic infiltrate and cell debris in the alveolar ducts with increased histiocytic infiltrate (1.B,D). Placenta showing in situ hybridization (RNAscope™ technology) for SARS-CoV-2 spike protein viral RNA (brown dots) resulted positive within the syncytiotrophoblast of multiple chorionic villi (1.E). Immunohistochemical (IHC) expression of SARS-CoV-2 nucleocapside (NC) protein in chorionic villi, resulting in a pattern of circumferential villous staining (1.G). Tissues from lungs at canalicular stage of the first fetus show expression of both SARS-CoV-2 spike protein viral RNA (brown dots, RNAscope) and of SARS-CoV-2 nucleocapsid (NC, IHC) (1.F,H). Original magnification: all x40.

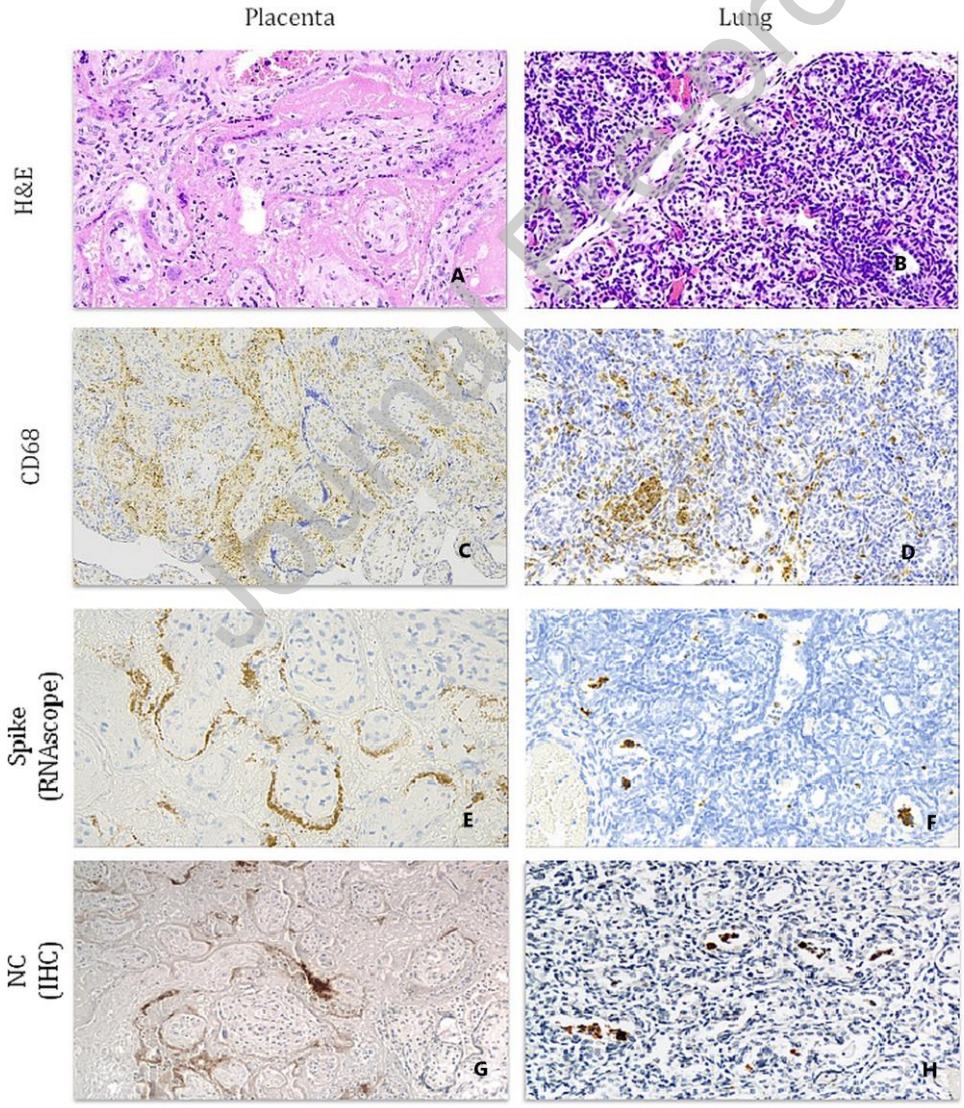

Table 1. SARS-CoV-2 Test Results

	SARS-CoV-2 RT-PCR frozen specimens	SARS-CoV-2 RT-PCR FFPE specimens	IHC ab-NC SARS-CoV-2	ISH Spike protein SARS-CoV-2	IHC ab-ACE-2 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
Hearth	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
Hearth	NA	POS	NEG	NEG	NEG
Kidney	NA	NEG	NEG	NEG	POS
Liver	NA	POS	NEG	NEG	NEG

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, reverse transcription-polymerase chain reaction; FFPE, formalin-fixed paraffin-embedded; IHC ab-NC, immunohistochemistry with antibody vs viral nucleocapsid protein; ISH Spike Protein, in situ hybridization with a RNA probe for the viral spike protein; IHC ab-ACE-2, immunohistochemistry with antibody vs angiotensin-converting enzyme 2 receptor; NA, not available; POS, positive; NEG, negative.

Table 2. Vertical transmission in first and second trimester of gestation, data from published papers.

Study	**GA**	**Covid - related Maternal Symptoms**	**Placental histological findings**	**SARS-CoV2 detection methods and findings**	
EVIDENCE OF SARS-CoV2 IN BOTH FETUS AND PLACENTA				**FETUS**	**PLACENTA**
Our case	21.4 twin	Asymptomatic	Chronic Intervillositis MVM features Chorioamniotis Funisitis	Lung + Kidney + Hearth + Liver +	Placenta + Cord + Membranes +
Valdespino – Vazquez et al. (7)	13 twin	Fever, myalgia, Headache, faringodynia	Placental infarctions, diffuse perivillous fibrin, active chronic intervillositis	Lung + Kidney +	Placentas +
EVIDENCE OF SARS-CoV2 ONLY IN PLACENTAL TISSUE				**FETUS**	**PLACENTA**
Baud et al. (4)	19 single	Fever, myalgia, diarrhoea, cough	Inflammatory infiltrates composed of neutrophils and monocytes in the subchorial space and unspecific increased intervillous fibrin deposition Funisitis	Lung - Liver - Thymus - Anus -	Placenta + Membranes +
Hosier et al. (5)	22 single	Fever, myalgia, diarrhoea, nausea, cough	Perivillous fibrin, histiocytic intervillositis	Lung - Heart -	Placenta + Cord +
Pulinx et al. (6)	24 twin	Fever and rynite at 22 weeks	Chronic intervillositis	Not available	Placentas + Amniotic fluid + Membranes -