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## **SARS-CoV-2 vertical transmission in a twin-pregnant woman: a case report**

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### Highlights

- The possibility of vertical transmission of SARS-CoV-2 is still debated
- Evidence of SARS-CoV-2 vertical transmission in a twin-pregnant woman
- Viral genome was found in the umbilical cord blood of both twins
- Placental histologic examination confirmed indirect signs of viral infection

### Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS- CoV-2) has affected millions of people around the world in the last years. Among susceptible patients, pregnant women seem to

be prone to have serious complications. The possibility of SARS-CoV-2 vertical transmission represents one of the most debated topics in the literature, providing inconclusive results.

We present a case of a confirmed vertical transmission in a monochorial diamniotic twin pregnancy complicated by a selective intrauterine growth restriction (sIUGR) and gestational diabetes. The analysis of different biological specimens identifies the presence of SARS-CoV2 genome in the umbilical cord blood of both twins and the placental histologic examination confirmed indirect signs of viral infection, supporting the hypothesis that a transplacental infection can occur.

Despite the devastating impact that SARS-CoV2 has worldwide, neonatal infections have been infrequently reported but they can occur under certain biologic conditions. A deep knowledge of the biological mechanisms underlying the risk of SARS-CoV-2 vertical transmission might be useful to understand the pathophysiological bases and the possible long-term implication of a mother-to-child vertical transmission.

**Keywords:** SARS-CoV-2 infection, Vertical transmission, Pregnancy, Placenta, Obstetric outcomes

## **Introduction**

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS- CoV-2) represents one of the most threatening infections in the last century, affecting millions of people, worldwide (World Health Organization, 2020) ([covid19.who.int](https://covid19.who.int)).

It exhibits a great variety of clinical manifestations, which can be worsened by pre-existing risk factors such as hypertension, diabetes, cardiovascular and respiratory diseases (Allotey et al., 2020; Huang et al., 2020).

Pregnant patients seem to be prone to serious complications, in fact, affected women are more likely to have adverse obstetric outcomes like preterm birth, intrauterine growth restriction, preeclampsia, and stillbirth (Allotey et al., 2020; Jamieson and Rasmussen, 2022; Porpora et al., 2021), compared to the uninfected pregnancies .

Different mechanisms are involved in the pathogenesis of SARS-COV2 vertical transmission. (Jamieson and Rasmussen, 2022; Sessa et al., 2022). International societies have proposed several antepartum, intrapartum, and postpartum strategies to contain the infection and accurately check the risk of fetal infection (El-Goly, 2021; Narang et al., 2020). In 2021, WHO prepared a scientific brief to describe a step-by-step process to screen for the possibility of vertical transmission, according to the timing of viral infection. Immediately after birth, they strongly suggest collecting nasopharyngeal swabs and blood samples from the newborn and subsequently searching for SARS-CoV2 RNA and IgG and IgM antibodies against viral antigens (World Health Organization, 2021). Furthermore, histologic examination of placental tissue and microscopic evaluation of vascular damage should be undertaken to assess indirect signs of viral infection (Sharps et al., 2020). In the light of the above, despite the increasing interest in the pathogenesis of SARS-CoV-2 vertical transmission, it remains a controversial issue and the available data on evidence and management of positive cases lead to inconclusive results (El-Goly, 2021; Narang et al., 2020; World Health Organization, 2021).

### **Case presentation**

We report the case of a 29-year-old twin-pregnant woman at 33 weeks, gravida 2, para 1, who was admitted to our Obstetric Emergency room, complaining of uterine contractions. She had a monochorial diamniotic twin pregnancy complicated by a selective intrauterine growth

restriction (sIUGR) and gestational diabetes type A1, according to Priscilla White's classification system (White, 1978)..

The patients did not report any respiratory symptoms and denied being previously vaccinated. According to the hospital guidelines, she underwent a real-time PCR (RT-PCR) nasopharyngeal swab, tested positive for SARS-CoV-2, and she was admitted into the Covid-19 obstetric ward.

### **Investigations**

At the admission, she presented a body temperature of 36.5°C, a blood pressure of 110/65 mm Hg, a pulse rate 80 bpm, and blood oxygen saturation of 98% in room air.

Ultrasound evaluation (US) showed fetus A with an estimated birth weight (EBW) of 1700 gr and normal Doppler velocimetry, and fetus B with an unequal placental sharing and a condition of sIUGR (EBW= 1300 gr) with abnormal middle cerebral artery pulsatility index (1.23, <5° percentile for the gestational age). Amniotic fluid and placental insertion were normal. Non-stress test (NST) was reactive with occasional uterine contractions.

Her vital signs, blood tests, arterial blood gas analyses NST and US were daily performed. Blood exams showed normal results, except for a mild anaemia and elevated C-reactive protein, whose values normalized during the hospitalization.

### **Treatment**

Since she did not suffer from any respiratory symptoms or other medical conditions, according to the indication of the infectious disease's specialist, she did not undergo any antiviral treatment. The arterial blood oxygenation was normal, so that she didn't require any non-invasive ventilation or any supplemental respiratory imaging.

She only received a daily injection of enoxaparin 4000 IU and 12 mg of intramuscular dexamethasone for 2 days to induce twins' lung maturation. Fetal wellbeing was checked by daily cardiotocography.

Ten days later, the patient exhibited painful uterine contractions and an emergency caesarean section was performed.

### **Outcomes and follow-up**

Two female newborns were delivered: the first weighed 1700 gr, she had an APGAR score of 8 and 9 at 1 and 5 minutes, respectively, and her cord blood pH was 7.22 with a base excess (BE) value of -6.8, the second girl had a weight of 1500 gr and an APGAR score of 7 and 8 at 1 and 5 minutes; the cord blood gas analysis showed a pH of 7.10 with a BE of -10.8.

The newborns were transferred to the neonatal intensive care unit (NICU). At the admission, they were stabilized by a neonatologist and two trained nurses. Three nasopharyngeal swabs were collected at 1 hour, 5 and 10 days after birth. All the swabs showed negative results. Conversely, the microbiological analysis of the placental tissues and the cord blood samples detected the presence of the RNA of SARS-CoV2 in these specimens. The laboratory findings are described in Table 1.

Histologic examination of the placenta revealed a chronic intervillitis and a diffuse placental malperfusion.

Placental and cord blood samples were clotted 60–90 minutes and then centrifuged for 10 minutes at 1300xg. The serum fractions were aliquoted and stored at –80°C until analysis, 10 cm of placental tissue (including maternal and fetal interface) was immediately collected after delivery and stored in formalin solution at room temperature.

Viral RNA was extracted from serum samples (200 $\mu$ L) by using the MOLgen Universal Extraction Kit (Adaltis, Italy), RNA extraction from placental tissue (30 mg) was performed, using the RecoverAll Total Nucleic Acid Isolation Kit (Invitrogen, Thermo Fisher Scientific, USA). The extracted RNA was amplified via RT-PCR by using the MOLgen SARS-CoV-2 RT-PCR Kit (Adaltis, Italy) (Bohn et al., 2020; Favaro et al., 2021). Three SARS-CoV-2 targets were identified: the N and E genes, specific for SARS-CoV-2, and the RNA-dependent RNA polymerase (RdRp) gene, present in all coronaviridae. Samples were considered positive when either the N or E genes, alone or together with the RdRp gene, were detected at  $\leq 38$  cycle threshold.

Maternal and cord blood specimens were screened for the presence of viral antibodies which showed positive concentration of anti-S antibodies (5.5 and 0.4 binding antibody units/ml (BAU/ml) respectively) and negative results of anti-N antibodies (0.33 and 0.08 U/ml respectively) (Table 1).

The patient's signs and symptoms were carefully checked during the following days, and the woman was discharged in good clinical conditions three days after the delivery, whereas the preterm twins underwent intensive examinations and were safely discharged in a month.

## Discussion

SARS-CoV-2 infection in pregnancy has been extensively studied while vertical maternal-fetal transmission is still under debate (Allotey et al., 2022; Sinaci et al., 2021). Allotey et al., in fact, assessed the contributing factors of SARS-CoV2 vertical transmission through a systematic review and meta-analysis including 472 studies with 18237 newborns from Covid-19 positive mothers. Their analysis suggested the rare occurrence of fetal infection, which appears to be associated with the severity of maternal Covid-19 symptoms (Allotey et al., 2022). The reported case suggests that vertical transmission can occur: the viral genome was found in both maternal



and cord blood, as well as placental tissue. Furthermore, the related histopathological alterations of the placenta, add more evidence to the transplacental passage of viral particles: in fact, we observed intense intervillitis and placental malperfusion. This can be explained by subchorionic end intervillous deposition of apoptotic cells which severely impair the integrity of placental interface (Baergen and Heller, 2020; Baud et al., 2020; Miranda et al., 2019; Shanes et al., 2020). The absence of viral particles in nasopharyngeal swabs from newborns suggests the hypothesis of a different viral tropism in infants and the possible viral testing to different sites (e.g., neonatal faecal and blood samples).

In addition, the high levels of maternal anti-S antibodies and low dosage of anti-N antibodies, could be related to the severity of the patient's disease. Szymczak also reported that the production of anti-N antibodies is strongly associated with severe symptoms, hinting at the theory that the more aggressive the disease is, the more effective the antibody response is found (Szymczak et al., 2021). A deep knowledge of the underlying biological mechanisms might be useful to understand the pathophysiological bases and the long-term implication of vertical transmission.

#### **Competing Interests**

The authors declare the absence of any commercial or financial relationships as a potential conflict of interest.

#### **Ethical Approval Statement**

The study was approved by the Ethical Committee (CE n. 6275/2021) of the Department of Maternal and Child Health and Urological Sciences, Sapienza, University of Rome. The patient provided written informed consent for all the established procedures.

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**Table 1.** Determination of Anti-S, Anti-N antibodies and SARS-CoV-2 RNA in maternal and umbilical cord blood samples, and SARS-CoV-2 RNA in placental tissues. BAU: binding antibodies unit \*gene E is not specific toward SARS-CoV-2 but is present in all Coronavirus; † Ct: Cycle threshold, positive if  $\leq 38$ .

### Samples

#### Maternal blood serum

	Antibodies Concentration
Anti-S Antibodies	5.5 BAU/ml
Anti N- Antibodies	0.3 U/ml
	PCR Results (Ct)
Gene N	negative
Gene E*	negative
RdRp	negative

#### Umbilical cord blood serum (Infant 1)

##### Antibodies Concentration

<b>Anti-S antibodies</b>	0.088 BAU/ml
<b>Anti-N antibodies</b>	0.4 U/ml

	<b>PCR Results (Ct)</b>
Gene N	37,000 <sup>+</sup>
Gene E*	33,871 <sup>+</sup>
RdRp	Negative

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**Umbilical cord blood serum (Infant 2)**


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	<b>Antibodies Concentration</b>
<b>Anti-S antibodies</b>	0.087 BAU/ml
<b>Anti-N antibodies</b>	0.4 U/ml

	<b>PCR results (Ct)</b>
Gene N	35,488 <sup>+</sup>
Gene E*	34,418 <sup>+</sup>
RdRp	negative

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**Placental tissues**


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	<b>PCR results (Ct)</b>
Gene N	35,582 <sup>+</sup>
Gene E*	negative
RdRp	negative

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