

## **A Possible Case of Vertical Transmission of SARS-CoV-2 in a Newborn with Positive Placental In Situ Hybridization of SARS-CoV-2 RNA.**

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### Abbreviations

<b>Abbreviation</b>	<b>Full term</b>
COVID-19	Coronavirus disease 2019
DOL	Day of life
HOL	Hours of life
ISH	In situ hybridization
MVM	Maternal vascular malperfusion
RNA	Ribonucleic acid
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
qRT-PCR	Quantitative reverse transcriptase-polymerase chain reaction

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## **Introduction**

Little is known about the effects of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as a virus and Coronavirus disease 2019 (COVID-19) as a disease on the pregnant mother and their infants. Moreover, no definitive evidence on whether SARS-CoV-2, a ribonucleic acid (RNA) virus, can be vertically transmitted from an infected mother to the unborn fetus.

Currently, there is no clear evidence of whether SARS-CoV-2 can be vertically transmitted. We report an infant born to a mother with COVID-19, who tested positive for SARS-CoV-2 quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) at 24, 48 hours of life (HOL) and on day of life (DOL) 7. Moreover, placental in situ hybridization (ISH) revealed the presence of SARS-CoV-2 RNA.

## **Methods**

A retrospective review of the maternal and infant's charts was performed to obtain clinical and laboratory data. Institutional review board approval and written informed consent from the mother were obtained. Starting from March 22<sup>nd</sup>, 2020, all pregnant women presenting to the Labor and Delivery Unit were tested for SARS-CoV-2 by using qRT-PCR of a nasopharyngeal swab. The infants of positive mothers were screened at 24 hours of life by a nasopharyngeal swab.

Placental pathology was performed by standard protocols with hematoxylin and eosin-stained samples. A CD68 immunohistochemical stain was performed on a block that was then forwarded to the Pathology Department of Massachusetts General Hospital.

Medical SARS-CoV-2 RNA ISH was done at the Pathology Department of Massachusetts General Hospital. RNAscope<sup>®</sup> 2.5 LS Probe- V-nCoV2019-S Cat No. 848568

and, RNAscope® 2.5 LS Reagent Kit-RED Cat No. 322150 Advanced Cell Diagnostic (ACD), on automated BondRx platform (Leica Biosystems) were used. We used 5 µm thick sections of FFPE biopsy tissue. All the steps were done on the BondRx machine, including baking for 1 hour at 60° C and counterstaining with hematoxylin. RNA unmarking is done using Bond Epitope Retrieval Solution 2 for 15 min at 95° C, followed by protease treatment for 15 min and probe hybridization for 2 hours. Signal was amplified by a series of signal amplification steps followed by color development in red using (Bond Polymer Refine Red Detection, Leica) in the format of red dots.

We used three sets of controls that were obtained from Massachusetts General Hospital. First, we used 122 historical “normal” controls from uncomplicated term deliveries pulled from the 2000-2004 database for the indication of maternal group B streptococcal positivity. Second, we included 130 “pathologic” controls that included term and late preterm newborns from a database of placentas from mothers with neonates with hypoxic-ischemic encephalopathy. Finally, for ISH probe specificity, we utilized 10 placentas of SARS-CoV-2 negative mothers. The 10 placentas included patients with RNA viral infections (Zika exposure, human immunodeficiency virus infection, and Hepatitis C infection), intrauterine fetal demise, histopathologies associated with congenital infections and coagulopathies such as high stage and grade acute chorioamnionitis, high grade maternal vascular malperfusion (MVM), high grade fetal vascular malperfusion, high-grade villitis of unknown etiology, chronic histiocytic intervillitis, and multiple intervillous thrombi.

## Results

An otherwise healthy, 32-year-old gravida 2 para 0 female presented at 35+6 gestational age with vaginal bleeding and contractions. She reported subjective fever, mild chills, fatigue, dysgeusia, and anosmia beginning 1 day before the presentation. Her prenatal care started in the first trimester; her perinatal serologies were not significant. A 2630 grams (56<sup>th</sup> percentile) female was delivered via an urgent cesarean section due to bleeding secondary to placenta previa on April 7<sup>th</sup>, 2020. The APGAR scores were 9 at both 1 and 5 minutes. The newborn displayed no signs of respiratory distress. The newborn length was 49.5 cm (89<sup>th</sup> percentile), and occipitofrontal circumference was 35 cm (97<sup>th</sup> percentile). The father had potential exposure as he works as a respiratory therapist at an intensive-care unit treating patients with COVID-19. However, the father was never symptomatic and was never tested before or during pregnancy. The maternal screen for SARS-CoV-2 nucleic acid from nasopharyngeal swab was positive on postpartum day 1.

The mother wore a surgical mask and a disposable, non-sterile isolation gown throughout her hospitalization. She washed her hands and chest with soap and water before breastfeeding or skin to skin care. The infant was kept inside a closed incubator, placed 6 feet away from the mother's bed in the same room. She fed her baby with formula and direct breastfeeding. On postpartum day 2, the mother started expressing her breast milk, and the nurse fed the baby.

The infant's nasopharyngeal qRT-PCR for SARS-CoV-2 was positive at 24 and 48 HOL. The infant's complete blood count, basic metabolic panel, and a C-reactive protein were all within normal limits. The Mother and infant remained afebrile, asymptomatic, and hemodynamically stable throughout the hospitalization. The infant was discharged with the mother on the DOL 4 with a daily telehealth visit plan up to DOL 14. The infant's nasopharyngeal qRT-PCR remained positive on DOL 7. He remained asymptomatic during

the daily follow-up period. Serologic studies COVID-19 IgM and IgG were not performed as testing facilities were not available. Up to our knowledge, no SARS-CoV-2 transmission to any other individual resulted from this case.

The gross examination of the 469-gram trimmed placenta was notable for a hypercoiled umbilical cord. The placental histopathology showed no inflammation (chronic villitis, intervillitis, or chorioamnionitis). However, it showed one central infarct with peripheral increased perivillous fibrin, a finding common in bleeding placenta previa. On routine stains, the infarcted area showed fetal placental vascular rupture and villous necrosis. A CD68 immunohistochemical stain showed subjective increased numbers of CD68 immunoreactive Hofbauer cells in the villous stroma (Figure 1).

ISH for SARS-CoV-2 RNA revealed a strong signal in the villous syncytiotrophoblast in a multifocal pattern (Figure 2). The placental signal was restricted to the syncytiotrophoblast, not present in villous stromal cells, Hofbauer cells, or villous endothelium.

### **Discussion:**

In this case, a neonate born to a mother with COVID-19 had positive nasopharyngeal qRT-PCR at 24, 48 HOL, and DOL 7. The infant remained afebrile and asymptomatic with normal hematologic and inflammatory markers. There was no evidence of growth restriction nor microcephaly. However, since the delivery time was close to the time of the maternal infection, we cannot rule out the risk of growth restriction and microcephaly in future cases. Although the mother wore personal protective equipment, the infant was not isolated immediately after delivery. The baby started breastfeeding at 1 HOL, and the first neonate nasopharyngeal qRT-PCR was done at 24 HOL. Moreover, the mother breast milk was not tested for SARS-CoV-2. Therefore, the neonate positive nasopharyngeal qRT-PCR may be

secondary to contamination with maternal breast milk. Thus, the possibility of postnatal exposure cannot be eliminated. However, the placental villous syncytiotrophoblast was positive for SARS-CoV-2 by ISH, suggesting vertical transmission.

There are approximately 5 thousand births per year in our center. Between March 22<sup>nd</sup> and July 31<sup>st</sup>, 2020, 1,463 mothers gave birth at our center, and all were universally screened for SAR-COV-2 at admission. Of the 1,463 mothers, 125 mothers (8.5%) had a positive SARS-CoV-2 nasopharyngeal qRT-PCR. The only positive newborn was the one described in our case report. Moreover, 53 placentas from positive mothers were tested by ISH for SARS-CoV-2 viral RNA. Only two placentas were positive. The first placenta was the one described in our report. The second placenta showed viral particles only within the endometrial glands but not in the syncytiotrophoblasts. The endometrial tissue is strictly maternal. Therefore, the viral presence does not reflect a vertical transmission. Accordingly, our data suggest a low probability of vertical transmission rate between positive mothers and their newborns.

Hecht *et al.* and Zhang *et al.* examined 19 and 74 SARS-CoV-2 exposed placentas, respectively [1,2]. There were no specific histopathologic features within the placentas of positive mothers compared with controls in both cohorts [1,2]. However, Shanes *et al.* examined 16 placentas of mothers with SARS-CoV-2 [3]. They reported an increase in the rate of features of MVM and intervillous thrombi compared with historical controls [3]. Intervillous thrombi and MVM are associated with maternal hypertensive disorders such as hypertension and pre-eclampsia. However, there was a negative association between pre-eclampsia and positive SARS-CoV-2 mothers in both Shanes *et al.* and Zhang *et al.* [1,2]. Therefore, more studies are needed to evaluate the relationship between MVM, placenta coagulopathies, and SARS-CoV-2.

Viruses can infect stromal cells, especially the resident macrophages (the Hofbauer cells), either via the receptors on the villous syncytiotrophoblast or via the breaks in the villous trophoblast covering of the villous stroma. Therefore, in our case, the strong signal of SARS-CoV-2 in the syncytiotrophoblast may represent vertical transmission. Chronic villitis and/or intervillitis are considered a histopathological hallmark of vertically transmitted viral infections. However, RNA viruses can infect the placenta without causing characteristic histopathology except for Hofbauer cell hyperplasia, chronic histiocytic intervillitis, and massive perivillous fibrin deposition [2]. In our case, the placental histopathology showed no chronic villitis, intervillitis, or chorioamnionitis. The lack of acute or chronic signs of placental inflammation in our case may be attributed to the lack of severe maternal systematic inflammatory response. However, the increased numbers of CD68 immunoreactive Hofbauer cells in the villous stroma may represent histiocytic intervillitis. Moreover, histiocytic intervillitis and Hofbauer cell hyperplasia were associated with other RNA viral placental infections, especially the ZIKA virus [4]. However, the lack of the SARS-CoV-2 signal in the Hofbauer cells necessitates further exploration of the possibility of maternal histiocytes carrying SARS-CoV-2.

Our report is limited by including only one single case with no PCR testing of the amniotic fluid or maternal and newborn serum. Moreover, the mother started to have symptoms just one day before delivery. Therefore, the vertical transmission may suggest that the mother was viremic during her incubation period. However, no clinical testing capability was available for testing the SARS-CoV-2 viral load of the mother or fetus's blood. More studies are needed to confirm the vertical transmissibility of SARS-CoV-2 and produce a consistent description of COVID-19 in newborns.



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## **Conflicts of Interest**

The authors have no conflicts of interest relevant to this article to disclose.

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## References:

1. Zhang P., Heyman T., Salafia C., et al. Detection of SARS-CoV-2 in placentas with pathology and vertical transmission [published online ahead of print, 2020 Aug 3]. *Am J Obstet Gynecol MFM* **2020**;100197. doi:10.1016/j.ajogmf.2020.100197.
2. Hecht JL, Quade B, Deshpande V, et al. SARS-CoV-2 can infect the placenta and is not associated with specific placental histopathology: a series of 19 placentas from COVID-19-positive mothers [published online ahead of print, 2020 Aug 2]. *Mod Pathol* **2020**;1-12. doi:10.1038/s41379-020-0639-4
3. Shanes ED., Mithal LB., Otero S., et al. Placental Pathology in COVID-19. *Am J Clin Pathol* **2020**;154(1):23-32. doi:10.1093/ajcp/aqaa089
4. Martines RB., Bhatnagar J., de Oliveira Ramos AM., et al. Pathology of congenital Zika syndrome in Brazil: a case series. *Lancet* **2016**;388(10047):898-904. doi:10.1016/S0140-6736(16)30883-2

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## Legends

**Figure 1** Placental villous tissue with (A) hematoxylin and eosin (H&E) staining and (B) immunohistochemical staining for CD68 for tissue macrophages (Hofbauer cells) within the distal villi. Scattered macrophages within the intervillous spaces are also present (200x magnification).

**Figure 2** Non-infarcted chorionic villi from placenta to illustrate SARS-CoV-2 RNA in syncytiotrophoblast. (A) In situ hybridization for SARS-CoV-2 RNA at 4X original showing clusters of positive red staining of villi. (B) In situ hybridization for SARS-CoV-2 RNA at 20X original demonstrating strong signal in syncytiotrophoblast in some but not all villi. (C) Hematoxylin and Eosin stained section of chorionic villi to identify syncytiotrophoblast (arrow) and vasculosyncytial membranes (arrowhead) 20X original.

Figure 1

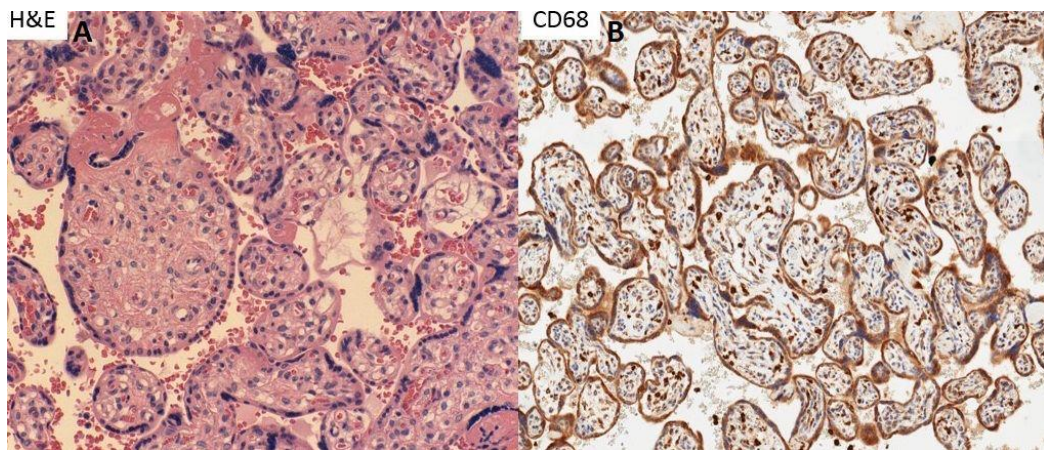
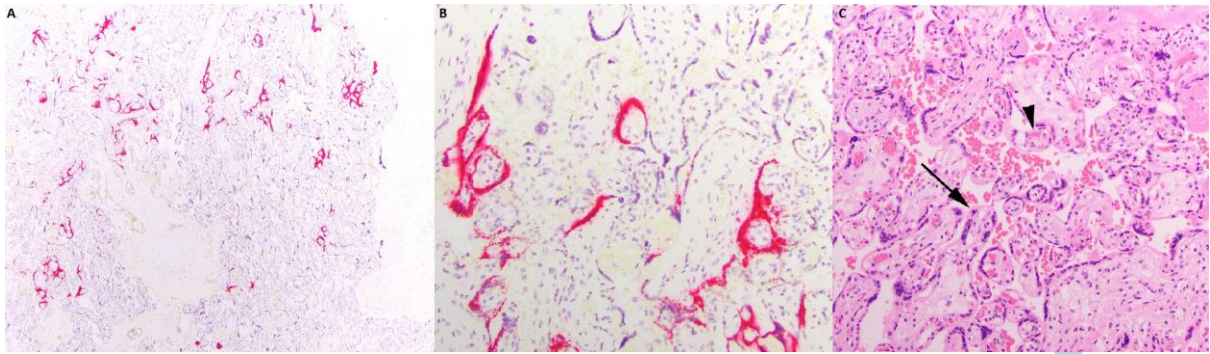


Figure 2



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