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Highlights

- SARS-CoV-2 was found in syncytiotrophoblast tissue between week 8 and 12 of pregnancy
- SARS-CoV-2 was found in fetal tissue between week 8 and 12 of pregnancy
- Mothers did not display any detectable viremia
- Realtime PCR results were confirmed by QuantiGene assay

SARS-CoV-2 vertical transmission during the first trimester of pregnancy in asymptomatic women

Running title: SARS-COV-2 VERTICAL TRANSMISSION DURING THE FIRST TRIMESTER

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ABSTRACT 150-200

Objective: It is now well established that in utero vertical SARS-CoV-2 transmission can occur during the late third trimester. However, little is known about other gestational ages. Recently, an increased risk of early miscarriage was reported in SARS-CoV-2 positive pregnant women. The objective of the present study is to evaluate the putative SARS-CoV-2 vertical transmission during the first trimester of pregnancy.

Design: This is an observational study on SARS-CoV-2-positive pregnant women during the first trimester. Fetal and syncytiotrophoblastic specimens were collected by hysterosuction from seventeen SARS-CoV-2-positive pregnant women who voluntarily terminated the pregnancy between week 8 and 12. We investigated the viral vertical transmission, by the means of SARS-CoV-2 RNA detection in fetus and syncytiotrophoblast, by two different techniques.

Results: Results suggest that SARS-CoV-2 vertical transmission is indeed possible during the first trimester in asymptomatic women. While maternal viremia was never detected, roughly 30% of the fetuses and 17% of the syncytiotrophoblasts resulted to be SARS-CoV-2-positive.

Conclusion: Indeed, SARS-CoV-2 is able to spread to the fetus through the syncytiotrophoblast. Concerningly, this happens in asymptomatic pregnant women as well. Long-term possible detrimental consequences on fetal development still need to be assessed. This should be taken into consideration in the management of pregnant women by implementing preventive strategies.

KEYWORDS: SARS-CoV-2; vertical transmission; first trimester; syncytiotrophoblast; fetus.

BACKGROUND

It is now well established that *in utero* vertical SARS-CoV-2 transmission can occur during the late third trimester (Fenizia et al., 2020; Vivanti et al., 2020). A systematic review of the cases reported in the literature estimated that, out of all the SARS-CoV-2 positive newborns, congenital transmission is ranging from 5.7 to possibly 12.2% (Raschetti et al., 2020). As specimens are easily accessible post-partum, many studies focused on the late third trimester to assess the risk of vertical transmission. However, little is known about other gestational ages.

The ability of SARS-CoV-2 of infecting a given tissue is directly correlated to the expression of viral host cell entry factors (Kumar et al., 2021; Wang et al., 2020). Among others, the two major host cell factors, which directly correlate with the ability of SARS-CoV-2 to successfully enter and infect cells, are the angiotensin-converting enzyme 2 (ACE2) and the transmembrane protease-serine 2 (TMPRSS2) (Hoffmann et al., 2020; Rossi et al., 2021). Interestingly, the placental expression of ACE2 and TMPRSS2 is higher during the first trimester, to then decrease overtime (Cui et al., 2021; Valdés et al., 2006). It is therefore tempting to speculate about a putatively higher risk of SARS-CoV-2 infection during such time. In the literature, just few cases of miscarried fetuses are reported, who then resulted to be SARS-CoV-2 positive themselves and/or their placenta, overall spanning from week 13 to 24 (Baud et al., 2020; Chamseddine et al., 2020; Hosier et al., 2020; Pulinx et al., 2020; Valdespino-Vázquez et al., 2021). The putative contribution of SARS-CoV-2 infection to such pregnancy losses needs to be carefully evaluated. Recently, a large study reported a higher risk of early miscarriage during the first trimester in SARS-CoV-2 infected pregnant women compared to the general, non-infected population (Balachandren et al., 2022). Moreover, those cases of miscarriages might represent the so-called “tip of the iceberg”, as the vertical transmission might occur more frequently early on during the pregnancy. The actual risk of

vertical transmission during the first trimester of pregnancy, and the possible detrimental consequences on fetal development, need to be promptly assessed.

In this scenario, we performed a study by testing the presence of the virus in syncytiotrophoblastic and fetal specimens on a cohort of seventeen SARS-CoV-2-positive women who voluntarily terminated the pregnancy during the first trimester.

MATERIAL and METHODS

Study population

This is an observation cross-sectional monocentric study that includes 17 pregnant women between week 8 and 12 who voluntarily terminated the pregnancy, in full compliance with the Italian law No. 194 of 22nd of May 1978. None of the pregnancy terminations was related to any medical condition. At the time of admission, they all resulted SARS-CoV-2 positive by PCR-testing the nasopharyngeal swab. All the subjects were enrolled between November 2020 and February 2021, with the Delta variant being the leading one at the time. Therefore, given the considered timeframe, none of the subject received any SARS-CoV-2 vaccination. All the subjects self-reported no prior SARS-CoV-2 infections. All women underwent clinical evaluation of vital signs and symptoms, laboratory analysis at admission. The therapeutic management was consequently tailored according to the clinical findings and national guidelines. Demographic and anthropometric characteristics and medical and obstetric comorbidities were recorded at enrolment through a customized data collection form, and summarized in table I. Briefly, all enrolled women were asymptomatic, and all pregnancies were singleton, with regular evolution for gestational age. Data accuracy was independently verified by two study investigators.

Specimen collection

Biological samples were collected during the termination procedure, which consisted in the hysterosuction intervention for all the enrolled subjects. In particular, full-thickness syncytiotrophoblasts and fetus biopsies were obtained. Bioptic samples were obtained in a sterile way by a dedicated operator. Moreover, a 10-ml maternal blood sample in EDTA was collected. Samples from obstetrics and gynecology units were immediately transferred to the dedicated laboratory to be readily processed.

Tissue processing

Collected syncytiotrophoblastic and fetal tissues were further processed only when clearly identifiable. The inner portion of syncytiotrophoblasts was isolated. Biopsies were manually dissected into few sections of approximately 2 mm^3. Such sections were then thoroughly homogenized and total RNA was isolated using the acid guanidinium thiocyanate–phenol–chloroform method (RNAbee, Duotech, Milan, Italy). As a result, RNA in RNase-free water was obtained.

Plasma samples were collected from the blood of all enrolled subjects. RNA was extracted by the Maxwell® RSC Instrument with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Promega, Fitchburg, WI, USA). As a result, RNA eluted in RNase-free water was obtained.

SARS-CoV-2 detection

Each RNA sample was both analyzed by real-time PCR and by QuantiGene technology (Thermo Fisher Scientific, Waltham, MA, USA).

Once RNA was reverse transcribed into cDNA, real-time PCR was performed on a CFX96 (Bio-rad, CA, USA) using TaqMan probes specifically designed to target two regions of the nucleocapsid (N) gene of SARS-CoV-2. For such application, we employed the 2019-nCoV CDC qPCR Probe Assay Emergency Kit (IDT, IA, USA), which also includes primers and probes that target the human RNase

P gene. A cycle threshold value (Ct-value) <37 was defined as a positive test result, according to the manufacturer's instructions.

Gene expression of 100 ng of RNA was performed by quantiGene Plex assay (Thermo Scientific, Waltham, MA, USA) which provides a fast and high-throughput solution for multiplexed gene expression quantitation based on Luminex technology, allowing the simultaneous measurement of 7 custom selected viral genes of interest in a single well of a 96-well plate. The quantiGene technology is not based on amplification of retro-transcribed samples, reducing samples handling.

All the procedures were carried out in accordance with the GLP guidelines adopted in our laboratory.

RESULTS

In order to address the risk of vertical transmission during the first trimester, we enrolled 17 unvaccinated SARS-CoV-2 positive asymptomatic/paucisymptomatic pregnant women between week 8 and 12 who voluntarily terminated the pregnancy.

Upon the medical procedure of pregnancy termination, abortive tissues were sampled. Specimens were processed and tested by real-time PCR, as previously described (Fenizia et al., 2020). Due to the collection procedure, samples were contaminated with maternal blood. Although it is unlikely, as SARS-CoV-2 viremia characterize the most severe clinical outcomes only (Hagman et al., 2022; Jacobs et al., 2021), we tested maternal blood as well to exclude a possible carry-over of the virus from such tissue. Upon collection by venipuncture before the clinical procedure, blood samples were processed and tested by real-time PCR. No plasma sample (0%) resulted SARS-CoV-2

positive. Stunningly, five out of the seventeen (29%) considered fetuses resulted SARS-CoV-2 positive, as well as three syncytiotrophoblasts (17%) (fig. 1). In two cases (n. 6 and 9) both the fetus and the syncytiotrophoblast tested positive, while in three cases (n. 2, 10 and 17) the fetus only. We found one case only (n.16) displaying SARS-CoV-2 positivity in the syncytiotrophoblast, but not the fetus. Moreover, this was the only case of discrepancy between the real-time PCR and the QuantiGene assay, probably due to the extremely low amount of virus. All the other samples samples tested by real-time PCR and by QuantiGene assay in parallel (fig 1A and 1B, respectively), resulted to be concordant. No significative differences were reached when comparing age, parity, gestational age or comorbidities between the pregnancies resulting in fetal/syncytiotrophoblast infection and the ones that did not, with the cohort-size as a limiting factor (data not shown).

DISCUSSION

Although preliminary, these data suggest that vertical transmission in the first trimester might be possible and, even more concerningly, it can happen in asymptomatic pregnant women as well.

The ability of SARS-CoV-2 to spread into the fetus is not an isolated case among viruses (Koyuncu et al., 2013). Indeed, among other pathogens, rubella virus and cytomegalovirus can be often vertically transmitted during the first trimester and commonly have severe consequences (Stegmann and Carey, 2002). On a broader picture, placental and fetal infection, or even just inflammation, relates to growth restriction, birth defects, bone marrow suppression, altered neurodevelopment, up to fetal demise (Megli and Coyne, 2022). It is still controversial whether SARS-CoV-2 pandemic has been going together with an increased miscarriage rate, as opposing data were reported (Cosma et al., 2021; Kazemi et al., 2021). However, the infection or the resulting inflammation might still have non-lethal detrimental effects, which may be hard to assess

now. I.e., the fetal neural system is intensely developing during the first trimester. It is already well documented that the complex network of neural circuits, the glia and neuron proliferation, migration, and synapse formation is affected by an inflammatory environment (Ganguli and Chavali, 2021; Han et al., 2021; Khan and Gomes, 2020). In parallel, it is now well understood that SARS-CoV-2 is able to penetrate the central nervous system and to develop an infection whose symptoms might last for months (Huang et al., 2021, p. 19; Wan et al., 2021). Indeed, part of the COVID-19-related symptoms pivots around the nervous systems, including the so-called Long-COVID (Carfì et al., 2020, p. 19; Davis et al., 2021; Huang et al., 2021). Long term consequences of SARS-CoV-2 on a developing fetal nervous systems still need to be assessed as, for example, cognitive skills developed in a span of time of years. It is probably too early to assess if SARS-CoV-2 might be "the newest spark" of TORCH (Muldoon et al., 2020).

How SARS-CoV-2 reaches the syncytiotrophoblast, especially in the absence of detectable viremia, is still an open question. The viral spreading throughout the different tissues/organs is not clearly identified. It may happen via systemic blood circulation, but in a rather transitory and hard-to-detect manner. On the other side, it has been proposed that SARS-CoV-2 could take advantage of different routes, such as the digestive tract (Scaldaferri et al., 2020; Trougakos et al., 2021), the central/peripheral nervous system (Trougakos et al., 2021; Wu et al., 2020), the lymphatic drainage system (Aguirre García et al., 2021; Bostancıklıoğlu, 2020; Xiang et al., 2021), together with the spreading through adjacent tissues (Zeng et al., 2022). We hypothesize that SARS-CoV-2 might reach the maternal uterine area by contiguity to the intestine, which is found to be positive in roughly 50% of SARS-CoV-2-infected subjects (Guo et al., 2021).

We observed that all the tested matching syncytiotrophoblast/fetus samples were not always concordant for SARS-CoV-2 positivity. We speculate that this apparent discrepancy might rely on biological reasons. Once the virus reaches the syncytiotrophoblast or the placenta, it spreads to

the fetus by either active viral production or just altered permeability, or both (Argueta et al., 2021; Fenizia et al., 2020; Hosier et al., 2020). It has been reported that placental infection is rather transitory and not highly productive (Colson et al., 2021; Tallarek et al., 2021), making viral detection harder. Such dynamics were observed in fully mature at-term placentas, which display different features from the syncytiotrophoblasts within week 12 included in our study, but no study was reported on this very same kind of specimen so far. An alternative explanation might rely on the sampling. SARS-CoV-2 does not spread through the whole syncytiotrophoblast/placenta, but rather infects distinct foci, putatively adding some inconsistency to the tissue sampling and possibly leading to a consequent under-estimation of the positive cases (unpublished observation), although we sampled and pooled multiple bioptic sections per each syncytiotrophoblast.

A caveat of the present study is the limited number of enrolled women, as these kinds of specimens are not easy to obtain. On the other side, as the PCR is somehow prone to be contaminated, on top of carefully applying all the good laboratory practices, we double-tested each sample with an additional different approach. In fact, the quantiGene assay is not based on amplification, as the PCR, and it was designed to target different regions, for an increased reliability. Moreover, the hysterosuction procedure is not prone to any kind of contamination, other than maternal blood. Together with the fact that there is no other study performed on such specimens during the first trimester of pregnancy, another strength of this work is that specimens were collected from asymptomatic mothers with no SARS-CoV-2-related complications. Indeed, this may be closer to the majority of the pregnancies and more informative on the extension of the actual risk of vertical transmission during the first trimester.

Little is known about the impact of SARS-CoV-2 infection in this scenario, which will probably be unraveled in next coming years. The present work provides the first insight on the putative risks of

SARS-CoV-2 vertical transmission during the first trimester, and it should be taken into consideration in the management of pregnant women by implementing preventive strategies, once again.

Conflict of Interest: The authors declare no conflict of interest.

Funding Source: The authors received no specific funding for this work.

Ethical Approval statement: The protocol was approved by the local Medical Ethical and Institutional Review Board (Milan, area 1, #154082020 – amendment 2020/EM/297). We obtained informed written consent from the mothers to perform the procedure and analysis, according to CARE guidelines and in compliance with the Declaration of Helsinki principles.

BIBLIOGRAPHY

Aguirre García MM, Mancilla-Galindo J, Paredes-Paredes M, Tiburcio ÁZ, Ávila-Vanzzini N. Mechanisms of infection by SARS-CoV-2, inflammation and potential links with the microbiome. Future Virology 2021;16:43–57. https://doi.org/10.2217/fvl-2020-0310.

Argueta LB, Lacko LA, Bram Y, Tada T, Carrau L, Zhang T, et al. SARS-CoV-2 Infects Syncytiotrophoblast and Activates Inflammatory Responses in the Placenta. BioRxiv 2021:2021.06.01.446676. https://doi.org/10.1101/2021.06.01.446676.

Balachandren N, Davies MC, Hall JA, Stephenson JM, David AL, Barrett G, et al. SARS-CoV-2 infection in the first trimester and the risk of early miscarriage: a UK population-based prospective cohort study of 3041 pregnancies conceived during the pandemic. Hum Reprod 2022:deac062. https://doi.org/10.1093/humrep/deac062.

Baud D, Greub G, Favre G, Gengler C, Jaton K, Dubruc E, et al. Second-Trimester Miscarriage in a Pregnant Woman With SARS-CoV-2 Infection. JAMA 2020. https://doi.org/10.1001/jama.2020.7233.

Bostancıklıoğlu M. SARS-CoV2 entry and spread in the lymphatic drainage system of the brain. Brain Behav Immun 2020;87:122–3. https://doi.org/10.1016/j.bbi.2020.04.080.

Carfì A, Bernabei R, Landi F, for the Gemelli Against COVID-19 Post-Acute Care Study Group. Persistent Symptoms in Patients After Acute COVID-19. JAMA 2020;324:603–5. https://doi.org/10.1001/jama.2020.12603.

Chamseddine RS, Wahbeh F, Chervenak F, Salomon LJ, Ahmed B, Rafii A. Pregnancy and Neonatal Outcomes in SARS-CoV-2 Infection: A Systematic Review. Journal of Pregnancy 2020;2020:e4592450. https://doi.org/10.1155/2020/4592450.

Colson A, Depoix CL, Dessilly G, Baldin P, Danhaive O, Hubinont C, et al. Clinical and in Vitro Evidence against Placenta Infection at Term by Severe Acute Respiratory Syndrome Coronavirus 2. Am J Pathol 2021;191:1610–23. https://doi.org/10.1016/j.ajpath.2021.05.009.

Cosma S, Carosso AR, Cusato J, Borella F, Carosso M, Bovetti M, et al. Coronavirus disease 2019 and first-trimester spontaneous abortion: a case-control study of 225 pregnant patients. American Journal of Obstetrics and Gynecology 2021;224:391.e1-391.e7. https://doi.org/10.1016/j.ajog.2020.10.005.

Cui D, Liu Y, Jiang X, Ding C, Poon LC, Wang H, et al. Single-cell RNA expression profiling of SARS-CoV-2-related ACE2 and TMPRSS2 in human trophectoderm and placenta. Ultrasound in Obstetrics & Gynecology 2021;57:248–56. https://doi.org/10.1002/uog.22186.

Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ, Re'em Y, et al. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. EClinicalMedicine 2021;38. https://doi.org/10.1016/j.eclinm.2021.101019.

Fenizia C, Biasin M, Cetin I, Vergani P, Mileto D, Spinillo A, et al. Analysis of SARS-CoV-2 vertical transmission during pregnancy. Nature Communications 2020;11:5128. https://doi.org/10.1038/s41467-020-18933-4.

Ganguli S, Chavali PL. Intrauterine Viral Infections: Impact of Inflammation on Fetal Neurodevelopment. Frontiers in Neuroscience 2021;15:1509. https://doi.org/10.3389/fnins.2021.771557.

Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal–oral transmission of SARS-CoV-2. Nat Rev Gastroenterol Hepatol 2021;18:269–83. https://doi.org/10.1038/s41575-021-00416-6.

Hagman K, Hedenstierna M, Rudling J, Gille-Johnson P, Hammas B, Grabbe M, et al. Duration of SARS-CoV-2 viremia and its correlation to mortality and inflammatory parameters in patients hospitalized for COVID-19: a cohort study. Diagnostic Microbiology and Infectious Disease 2022;102:115595. https://doi.org/10.1016/j.diagmicrobio.2021.115595.

Han VX, Patel S, Jones HF, Nielsen TC, Mohammad SS, Hofer MJ, et al. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. Transl Psychiatry 2021;11:1–12. https://doi.org/10.1038/s41398-021-01198-w.

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020;181:271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052.

Hosier H, Farhadian SF, Morotti RA, Deshmukh U, Lu-Culligan A, Campbell KH, et al. SARS-CoV-2 infection of the placenta. J Clin Invest 2020. https://doi.org/10.1172/JCI139569.

Huang C, Huang L, Wang Yeming, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. The Lancet 2021;397:220–32. https://doi.org/10.1016/S0140-6736(20)32656-8.

Jacobs JL, Bain W, Naqvi A, Staines B, Castanha PMS, Yang H, et al. SARS-CoV-2 Viremia is Associated with COVID-19 Severity and Predicts Clinical Outcomes. Clin Infect Dis 2021:ciab686. https://doi.org/10.1093/cid/ciab686.

Kazemi SN, Hajikhani B, Didar H, Hosseini SS, Haddadi S, Khalili F, et al. COVID-19 and cause of pregnancy loss during the pandemic: A systematic review. PLOS ONE 2021;16:e0255994. https://doi.org/10.1371/journal.pone.0255994.

Khan S, Gomes J. Neuropathogenesis of SARS-CoV-2 infection. ELife 2020;9:e59136. https://doi.org/10.7554/eLife.59136.

Koyuncu OO, Hogue IB, Enquist LW. Virus Infections in the Nervous System. Cell Host Microbe 2013;13:379–93. https://doi.org/10.1016/j.chom.2013.03.010.

Kumar A, Narayan RK, Prasoon P, Kumari C, Kaur G, Kumar Santosh, et al. COVID-19 Mechanisms in the Human Body—What We Know So Far. Frontiers in Immunology 2021;12.

Megli CJ, Coyne CB. Infections at the maternal–fetal interface: an overview of pathogenesis and defence. Nat Rev Microbiol 2022;20:67–82. https://doi.org/10.1038/s41579-021-00610-y.

Muldoon KM, Fowler KB, Pesch MH, Schleiss MR. SARS-CoV-2: Is it the newest spark in the TORCH? J Clin Virol 2020;127:104372. https://doi.org/10.1016/j.jcv.2020.104372.

Pulinx B, Kieffer D, Michiels I, Petermans S, Strybol D, Delvaux S, et al. Vertical transmission of SARS-CoV-2 infection and preterm birth. Eur J Clin Microbiol Infect Dis 2020:1–5. https://doi.org/10.1007/s10096-020-03964-y.

Raschetti R, Vivanti AJ, Vauloup-Fellous C, Loi B, Benachi A, De Luca D. Synthesis and systematic review of reported neonatal SARS-CoV-2 infections. Nature Communications 2020;11:5164. https://doi.org/10.1038/s41467-020-18982-9.

Rossi ÁD, de Araújo JLF, de Almeida TB, Ribeiro-Alves M, de Almeida Velozo C, Almeida JM de, et al. Association between ACE2 and TMPRSS2 nasopharyngeal expression and COVID-19 respiratory distress. Sci Rep 2021;11:9658. https://doi.org/10.1038/s41598-021-88944-8.

Stegmann BJ, Carey JC. TORCH Infections. Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections. Curr Womens Health Rep 2002;2:253–8.

Tallarek A-C, Urbschat C, Fonseca Brito L, Stanelle-Bertram S, Krasemann S, Frascaroli G, et al. Inefficient Placental Virus Replication and Absence of Neonatal Cell-Specific Immunity Upon Sars-CoV-2 Infection During Pregnancy. Frontiers in Immunology 2021;12.

Trougakos IP, Stamatelopoulos K, Terpos E, Tsitsilonis OE, Aivalioti E, Paraskevis D, et al. Insights to SARS-CoV-2 life cycle, pathophysiology, and rationalized treatments that target COVID-19 clinical complications. Journal of Biomedical Science 2021;28:9. https://doi.org/10.1186/s12929-020-00703-5.

Valdés G, Neves LAA, Anton L, Corthorn J, Chacón C, Germain AM, et al. Distribution of Angiotensin-(1-7) and ACE2 in Human Placentas of Normal and Pathological Pregnancies. Placenta 2006;27:200–7. https://doi.org/10.1016/j.placenta.2005.02.015.

Valdespino-Vázquez MY, Helguera-Repetto CA, León-Juárez M, Villavicencio-Carrisoza O, Flores-Pliego A, Moreno-Verduzco ER, et al. Fetal and placental infection with SARS-CoV-2 in early pregnancy. Journal of Medical Virology 2021;93:4480–7. https://doi.org/10.1002/jmv.26965.

Vivanti AJ, Vauloup-Fellous C, Prevot S, Zupan V, Suffee C, Do Cao J, et al. Transplacental transmission of SARS-CoV-2 infection. Nature Communications 2020;11:3572. https://doi.org/10.1038/s41467-020-17436-6.

Wan D, Du T, Hong W, Chen L, Que H, Lu S, et al. Neurological complications and infection mechanism of SARS-CoV-2. Sig Transduct Target Ther 2021;6:1–16. https://doi.org/10.1038/s41392-021-00818-7.

Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA 2020;323:1843–4. https://doi.org/10.1001/jama.2020.3786.

Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, et al. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun 2020;87:18–22. https://doi.org/10.1016/j.bbi.2020.03.031.

Xiang Q, Feng Z, Diao B, Tu C, Qiao Q, Yang H, et al. SARS-CoV-2 Induces Lymphocytopenia by Promoting Inflammation and Decimates Secondary Lymphoid Organs. Frontiers in Immunology 2021;12.

Zeng C, Evans JP, King T, Zheng Y-M, Oltz EM, Whelan SPJ, et al. SARS-CoV-2 spreads through cell-to-cell transmission. PNAS 2022;119. https://doi.org/10.1073/pnas.2111400119.

FIGURE LEGEND

A

	MB	FT	ST
1	-	-	-
2	-	31.97	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	32.20	30.75
7	-	-	-
8	-	-	-
9	-	32.68	28.85
10	-	32.69	-
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	-	32.86	-

B

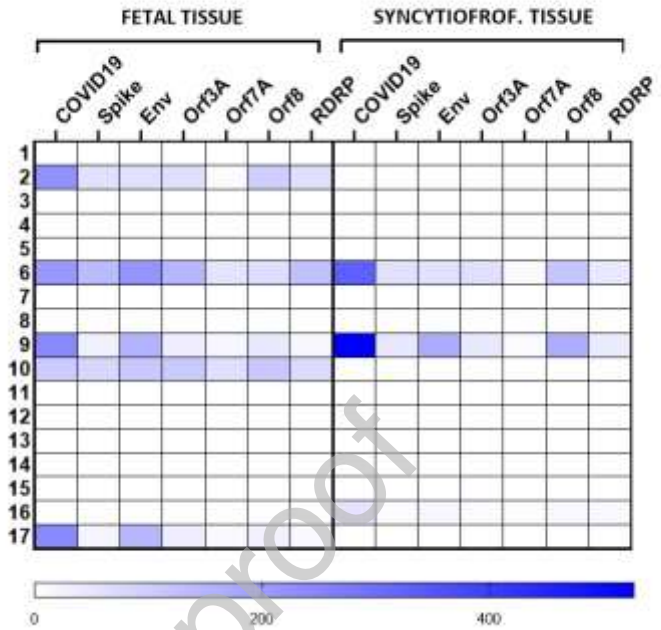

Fig. 1

Figure 1. SARS-CoV-2 detection for the 17 enrolled subjects. A) Real-time PCR Ct values are reported in panel A (MB-maternal blood. FT-fetal tissue. ST- syncytiotrophoblast tissue). B) QuantiGene assay on fetal and syncytiotrophoblastic tissues. For each of the viral targets, values are expressed as fluorescence arbitrary units.

Table I. Study population.

	Total study population (*n* = 17)
Age, years, median (range)	34 (27 – 39)
Gestational age at admission, median (range)	10 (8 – 12)
RT-PCR assay of a nasopharyngeal swab	
Positive, *n* (%)	17 (100)
BMI, kg/m^2, median (range)	22 (18 – 31)
Known sick contact, *n* (%)	1 (6)
Smoking, *n* (%)	2 (12)
Ethnicity, Caucasian, *n* (%)	9 (53)
Chronic comorbidity, *n* (%)	1 (6)
Obesity, n (%)	1 (6)
Parity, nulliparous, *n* (%)	4 (24)
Symptomatic Patients, n (%)	0 (0)