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Intrauterine vertical SARS-CoV-2 infection: a case confirming transplacental transmission followed by divergence of the viral genome

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SHORT RUNNING TITLE

Intrauterine vertical transmission of SARS-CoV-2

TWEETABLE ABSTRACT

COVID-19 can lead to intrauterine SARS-CoV-2 transmission with placental dysfunction and foetal distress.

KEY WORDS

Caesarean section, COVID-19, intrauterine transmission, hypoxia, placental insufficiency, SARS-CoV-2, transplacental transmission, vertical transmission

CASE

A 27-year-old woman (gravida 2, para 1) was transported to the regional university hospital in gestational week (GW) 34 + 4 due to a three-day history of fever, abdominal pain and reduced foetal movements. She had developed a dry cough one day prior to the admission (Figure S1).

The woman, was slightly overweight (BMI 27 kg/m^2) but otherwise healthy. She had normal antenatal check-ups and an obstetric ultrasound at GW 32 + 2 showed a normal foetal weight deviation of +8%[1].

At admission, the patient was promptly isolated in a negative pressure room at the delivery unit and standard operating procedures and personal protective equipment (PPE) were used[2]. A combined nasopharynx (NPH) throat swab for SARS-CoV-2 using real time reverse transcriptase quantitative polymerase chain reaction (RT qPCR) was obtained and normal vital parameters (apart from fever 38.3 degrees Celsius) were registered. The admission cardiotocograph (CTG) test showed reduced baseline variability, absence of accelerations with recurrent prolonged, and late decelerations (Figure S2). In light of the pathological CTG pattern, the obstetric team made the prompt decision to deliver the patient by an immediate caesarean section (CS). An uncomplicated CS was performed in an operating theatre with negative pressure in line with the international recommendations for COVID-19[2]. The total blood loss was 200 mL. The amniotic fluid was of normal amount and there were no signs of meconium staining or premature rupture of the amniotic membranes.

The neonate showed no initial signs of spontaneous breathing and was ventilated by neonatal staff in a separate room. A maximum of 80% supplemental oxygen was needed to maintain adequate saturation. At six minutes of age, the neonate established spontaneous breathing and continuous positive airway pressure (5 cm H_2O) was maintained for an additional 24 minutes, whereafter further ventilatory support was not needed. At one minute of age, the neonate had an Apgar score of 1 (heart rate = 1, remaining items = 0), at five minutes of age Apgar 4 (heart rate = 2, muscular tonus = 1, reflex irritability = 1, remaining items = 0), and at ten minutes of age Apgar 8 (heart rate = 2, respiratory activity = 2, skin colour = 1, muscular tonus = 2, reflex irritability = 1). Validated umbilical cord blood gases[3] showed a cord arterial pH of 7.20 and venous pH of 7.22.

Cord arterial lactate was 11 mmol/L whilst cord venous lactate was 10.1 mmol/L. Figure S1 illustrates the timeline of events for mother and child.

After the CS, the mother was isolated in the postpartum ward and the NPH/throat swab taken upon admission returned positive for SARS-CoV-2. Analysis of maternal blood was also RT qPCR positive for SARS-CoV-2. Serology from the day of delivery revealed that the mother was weakly positive for immunoglobulin (Ig) M and negative for IgG. Along with lymphocytopenia (0.7 $x10^9$/L) and thrombocytopenia (98 $x10^9$/L); inflammatory markers including c-reactive protein (36 mg/L), ferritin (340 µmol/L) and lactate dehydrogenase (9.5 µkat/L) were found to be elevated. The clinical condition of the mother improved and she was discharged four days after delivery. Thromboprophylaxis (Tinzaparin 4500 IE subcutaneously once daily) for a total six weeks postpartum was prescribed in accordance with National Guidelines in place at the time[4]. By day 11 postpartum, the mother was seropositive for anti-SARS-CoV-2 IgM and IgG. Breast milk analyzed day 14 postpartum was RT qPCR negative for SARS-CoV-2, and further, at day 35 postpartum, negative for anti-SARS-CoV-2 total immunoglobulin.

The neonate in the current case had no contact with any family member, including the mother, during the first 60 hours of life. Since neither skin-to-skin care nor any other contact with the mother occurred, the neonate was regarded as non-infected. In accordance with national guidelines at the time[4], the neonate was tested for COVID-19 using a NPH swab 48 hours after delivery. This test returned positive for SARS-CoV-2 and the neonate was then regarded as contagious. Infection control routines were initiated to investigate a potential COVID-19 breakout at the neonatal ward and to rule out the possibility of postpartum transmission. All staff that had tended to the neonate (n=27) and all nearby patients (n=4) were tested by NPH swab and SARS-CoV-2 RT qPCR returned negative in all cases (data not shown). Symptom surveillance in this group was continued for a further 14 days but no COVID-19 positive cases were discovered during this time.

The neonate was transferred and united with the mother at the postpartum ward isolation room at DOL 3 (60 hours after birth). Breastfeeding was thereafter initiated and the neonate did not receive any breastmilk before this time point. Repeated RT qPCR analyses showed the lowest neonatal CT-value at DOL 5 where after a gradual increase was seen. By DOL 20, SARS-CoV-2 was not detectable in NPH or throat swabs (Table S2). Serology revealed that the neonate was

anti-SARS-CoV-2 IgG negative at DOL 7 (IgM not analysed due to lack of material). At DOL 14, IgM was positive and IgG still negative and at DOL 20, the neonate was both IgM and IgG seropositive.

Viral genome sequencing

To determine the genetic clade and to fully investigate the viral genetic similarities, virus isolates from the mother (NPH/throat swab obtained on the day of delivery), and neonate (NPH swab obtained at 48 hours of age, labelled DOL 2, and further at DOL 5) as well as from placental tissue, were sent to the Public Health Agency of Sweden for whole-genome sequencing. Next-generation sequencing of samples produced several full length 29 903bp, SARS-CoV-2 genomes, all belonging to the genetic clade 20B/GR/B.1.1[5] (Table S3). All four sequences showed high identity. Further sequencing data analysis identified 12 variant positions in the sequences from isolates of the mother and placenta compared to the SARS-CoV-2 reference genome (NC_045512). These variants were also present in the sequences of the neonatal isolates. Notably, an additional variant, A107G, was identified in the neonate samples but only present in 67 and 80%, respectively, of the sequences.

Placental pathology

The placenta was easily detached from the uterus during the CS. The remaining umbilical cord stump had a central insertion, was 9 cm long with a diameter of 1 × 1.5 cm and contained three vessels. The membranes had normal colour without signs of meconium staining. The trimmed weight of the placental disc was 342 grams, within the 10th to 90th percentile for GW 34+0 to 34+6[6]. At gross sectioning, fibrinoid depositions were evident as glistening white-grey-pink confluent lesions, encompassing approximately 50% of the total placental volume (Figure 1A).

Microscopic examination confirmed the presence of confluent intervillous fibrinoid depositions accompanied by denudation of the villi from trophoblasts and syncytiotrophoblasts with dislocated syncytiotrophoblasts visible in the fibrinoid (Figure 1 B-C). There were multiple regions of dense intervillous infiltrates of neutrophilic granulocytes and macrophages (Figure 1 D). The areas devoid of intervillous fibrinoid depositions frequently showed chorangiosis (Figure 1 E). Immunohistochemistry confirmed that the inflammatory cell component of the intervillisotis was

dominated by myeloperoxidase positive granulocytes and CD68 positive macrophages with sparse amounts of CD3 and CD20 positive lymphocytes (Figure 1 F-G).

Immunohistochemical detection of SARS-CoV-2 nucleoprotein was strongly positive in the cytoplasm and nucleus of villous cytotrophoblasts and syncytiotrophoblasts in areas with intervillositis and fibrinoid depositions, with some positive staining in the villous stromal cells (Figure 1 H-J). In contrast, SARS-CoV-2 nucleoprotein staining was focal or absent in most but not all areas devoid of intervillositis (Figure 1 K-L). Additionally, presence of ribonucleic acid (RNA) virus was confirmed in both cytotrophoblasts and syncytiotrophoblasts by *in-situ* staining for double stranded RNA (Figure 1 M). There were no signs of villitis or inflammation in the membranes or umbilical cord. Immunohistochemistry for SARS-CoV-2 nucleoprotein was absent or showed faint signal in the amniotic membranes and the foetal chorionic vessels.

DISCUSSION

Vertical transmission is one of the major complications of viral diseases during pregnancy[7]. Pregnant women are more likely to need intensive care treatment related to COVID-19 as compared to non-pregnant women of reproductive age[8]. COVID-19 infection has also been associated with a higher rate of preterm birth, preeclampsia, CS, foetal vascular malperfusion, premature foetal membrane rupture and perinatal death[9, 10]. A number of reports have suggested vertical transmission[11], but to the best of our knowledge, only Vivanti et al.[12], Fenizia et al.[13] and Correia et al.[14] have convincingly reported cases of transplacental SARS-CoV-2 transmission (Table S1).

Several studies have found SARS-CoV-2 in placental tissue, amniotic fluid and in cord blood[15-17] however, vertical transmission seems to be a rare complication of COVID-19 in pregnancy. SARS-CoV-2 may be physically blocked by the placental barrier defense mechanisms, combated by immune-regulatory molecular pathways or, in the case of placental infection, immunomodulatory mechanisms may soften the cytokine storm associated with severe COVID-19 disease. This reduction in cell and tissue damage has been postulated to potentially reduce the risk of SARS-CoV-2 transmission to the foetus[18]. The placenta is therefore of key interest in understanding perinatal transmission. For SARS-CoV-2, angiotensin-converting enzyme 2 (ACE2) is the undisputed receptor for cellular entry. Co-expression of the protease, TMPRSS2, is also required to cleave the spike (S) protein on SARS-CoV-2, mediating ACE2 binding. Studies have shown low levels of ACE2 and TMPRSS2 mRNA in the syncytiotrophoblast and extravillous trophoblast cells of the placenta[15, 19] and, due to negligible co-transcription of ACE2 and TMPRSS2 in the placenta, it has been argued that ACE2 is not a likely path of vertical transmission for SARS-CoV-2[20]. In contrast, receptors for Zika virus and cytomegalovirus, which cause congenital infections, are abundant in placental cell types. To the best of our knowledge, no studies have demonstrated SARS-CoV-2 cellular entry via ACE2 binding nor replication within placental cells[21].

Other studies have suggested that placental inflammation may play a central role in the transmission of SARS-CoV-2. Lye et al.[22] reported that ACE2 expression was increased in placentas that experienced chorioamnionitis. The authors proposed the possibility of vertical transmission by way of SARS-CoV-2 carrying immune cells infiltrating placentas complicated

with chorioamnionitis. Antibodies can also facilitate the transport of viruses across the placenta by Fc receptor binding. SARS-CoV-2 infection of placental macrophages (Hofbauer cells) has only been identified in a single patient from one clinical report, suggesting that this may be a rare mechanism of SARS-CoV-2 transmission[23]. There is additional evidence, as described by Yoel Sadovsky et al.[24] on how miRNA in the placenta can protect against viral infections.

In the current study, viremia may have led to more severe maternal disease with rapid deterioration of placental function secondary to inflammation. Viremia in the blood is rare. According to Wang et al.[25], SARS-CoV-2 RNA was found in only 1% of blood samples taken from COVID-19 patients. It may therefore be argued that the placenta is highly active in preventing SARS-CoV-2 vertical transmission and although the exact mechanisms of placental defense against the virus are yet to be elucidated, inflammation may play a central role.

The mother presented with classic COVID-19 symptoms including fever and a dry cough[26] but abdominal pain and reduced fo etal movements were also reported. Similar to previous reports, we observed that the clinical condition of the mother improved rapidly after delivery[27, 28]. The mother also presented with elevated concentrations of several acute phase proteins including ferritin, procalcitonin and c-reactive protein, indicating systemic inflammation[29]. In addition, at the time of delivery, SARS-CoV-2 RNA was found in the maternal blood and RT qPCR indicated the highest viral load within the placenta. RT qPCR does not produce an exact quantification of viral load as different materials are analyzed. However, the cycle threshold (CT) values were clearly the lowest in the placental specimen and histopathological placental analyses indicated high levels of SARS-CoV-2. Viral protein was found in the villous cytotrophoblasts, in the syncytiotrophoblasts and massive perivillous fibrin deposits covered over 50% of the placenta. The placental histopathological changes seen in this case are similar to several previous reports on SARS-CoV-2, as well as SARS-CoV-1 and MERS-CoV[12, 23, 30, 31].

The neonate in the current case suffered from transient asphyxia attributed to intrauterine hypoxia secondary to placental dysfunction. This was signaled by the pathological CTG registering and validated umbilical cord blood gases revealed a cord arterial and venous pH below normal median reference values for 34 weeks of gestational age[3]. More notably, the neonate had abnormally high cord arterial and venous lactate values which clearly indicated a hypoxic insult[32]. Following

initial resuscitation, only standard supportive care of prematurity was needed. No evident signs of COVID-19 were observed and repeated RT qPCR testing revealed the lowest CT-values at DOL 5, suggestive of the highest viral load in the upper respiratory tract at this time point since a lower CT level implies a greater amount of SARS-CoV-2 nucleic acid. The CT-values later increased and by DOL 20, SARS-CoV-2 RNA was not detectable. Consistent with the observed viral clearance, neonatal IgM and IgG seroconversion was found. Previous knowledge of immunoglobulin transfer during pregnancy along with new data from the current COVID-19 pandemic confirm that anti-SARS-CoV-2 IgG can pass through the placental barrier whilst IgM does not[33, 34]. In the current case, maternal serum was weakly positive for IgM and negative for IgG at the day of delivery. Further, the neonate was seronegative for IgG at DOL 7 (IgM not tested due to lack of material) and we conclude that transplacental transfer of anti-SARS-CoV-2 immunoglobulin was not likely and that the neonate seroconverted by its own means. The possibility of the neonate acquiring COVID-19 postpartum was ruled out by vigorous testing of all staff that had been in contact with the neonate during the first 48 hours of life, as well as surrounding patients and their attendees. Secondary symptom surveillance for two weeks revealed no new cases.

To fully determine viral genome similarities between the mother, neonate and the placenta, whole-genome sequencing was performed. All four isolates revealed 29 903bp SARS-CoV-2 genomes, belonging to the genetic clade 20B/GR/B.1.1. Further analysis of the sequencing data showed that the isolate from mother and placenta had 11 single-nucleotide polymorphisms (SNPs) and one multiple-nucleotide polymorphism (MNP) differences compared to the reference Wuhan genome of SARS-CoV-2. Interestingly, the two neonatal isolates, from DOL 2 and DOL 5, both had a mixed population of the virus. In addition to a population of the virus with the same genotype as the isolates from the mother and placenta, the neonate isolates contained another population of virus (80% identical to maternal isolates on DOL 2 and 67% in DOL 5) with an additional SNP, e.g. A107G. Intrapatient genetic variation has previously been described in both MERS-CoV and SARS-CoV-2[35, 36]. To the best of our knowledge, this is the first case of ongoing genetic change in neonatal COVID-19 in the unique setting of intrauterine transmission.

The SARS-CoV-2 genome has been reported to have an evolutionary rate of around $8 \times 10{-}4$ substitutions per site per year[37, 38]. Although rapid compared to its hosts' evolutionary rate or

bacteria, is it considered slow for an RNA virus. Due to this and previous studies in literature, we did not expect the genome to diverge. As such, even the small divergence seen between the two patients is worth noticing. Several explanations can explain the genetic divergence of the virus in the current case. Genetic drift, i.e. a genetic variant that happened to spread without being actively selected for. It may have occurred by random mutation in the placenta with replication in parallel to the original variant. We have no data here distinguishing drift from selection. This would require functional studies and more cases where this variant is demonstrated to be enriched in the placenta compared to the mother. Secondly, and more likely, transfer from mother to neonate spurred evolution, due to change in the environment. Overall however, all virus isolates from mother, neonate and the placenta, displayed a clear similarity and shared a majority of the SNP's.

Given these genetic findings and the series of events presented above, along with the marked placental pathology and the high viral load, it can therefore be concluded that the neonate was infected *in utero*. The two main clinical lessons that can be learnt from the current case are; I) Intrauterine vertical transmission is an uncommon complication of COVID-19 during pregnancy which may lead to placental dysfunction and clinical consequences for the newborn, and II) intrauterine SARS-CoV-2 transmission may not necessarily lead to severe neonatal outcome.

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DISCLOSURE OF INTEREST

The authors declare no conflicts of interest.

CONTRIBUTION TO AUTHORSHIP

M.Z, A.M.H and P.T conceived the project, performed the literature search, prepared the tables, figures, merged and interpreted all the data and wrote the manuscript draft. L.J, M.Z and A.M.H managed the mother. M.Z interpreted the maternal clinical picture and laboratory tests. P.T, J.S, and O.A managed the neonate, interpreted the neonatal clinical picture and laboratory tests. A.S.S interpreted the SARS-CoV-2 diagnostic data. M.L.K and O.K.L performed the whole genome sequencing and data analysis. S.R.H, D.G.N, M.L.K and O.K.L helped in data interpretation and revision of the manuscript. D.G.N performed the pathological examination, prepared the figures and co-authored the text. All authors critically reviewed the manuscript for important intellectual content and approved it in its final version.

DETAILS OF ETHICS APPROVAL

The mother and father have provided written informed consent to publication, available upon request. The case study was performed in agreement with principles of the Declaration of Helsinki.

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DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

This article has a Video Abstract presented by Mehreen Zaigham.

https://www.dropbox.com/s/ozrse6ujb0ucnab/IMG_9564.MOV?dl=0

REFERENCES

1. Marsál K, Persson PH, Larsen T, H Lilja, A Selbing, B Sultan. Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr* 2020; **85**(7): 843-8.
2. Coronavirus (COVID-19) Infection in Pregnancy. Information for healthcare professionals (online)Version 7: Published Thursday 9 April 2020. Assessed September 25, 2020. Available from: https://www.rcog.org.uk/globalassets/documents/guidelines/2020-04-09-coronavirus-covid-19-infection-in-pregnancy.pdf
3. Zaigham M, Källen K, Olofsson P. Gestational age-related reference values for Apgar score and umbilical cord arterial and venous pH in preterm and term newborns. *Acta Obstet Gynecol Scand* 2019; **98**(12):1618-23.
4. Swedish Society for Obstetrics and Gynecology and Swedish Neonatal Society (Internet) recommendations for care of pregnant women and neonates born to women with confirmed or suspected COVID-19. (online)Version 2, updated 2020-04-05. Available from: https://neo.barnlakarforeningen.se/wp-content/uploads/sites/14/2020/03/Rekommendation-om-handläggning-av-gravida-och-barn-till-kvinnor-med-verifieradelsannolik-Covid-19_ver-2_200405.pdf
5. Global Initiative on Sharing All Influenza Data (GISAID). Genomic epidemiology of hCoV-19 EpiCoV (online) (Cited 2020-10-09) Available from: https://www.epicov.org/
6. Kraus F, Redline R, Gersell D, Nelson M, Dicke J Placental Pathology. American Registry of Pathology 2005.
7. Pereira L Congenital Viral Infection: Traversing the Uterine-Placental Interface. *Annu Rev Virol* 2018; **5**: 273-99.
8. Ortiz-Prado E, Simbana-Rivera K, Gomez-Barreno L, Rubio-Neira M, Gauman L, Kyriakidis N et al. Clinical, molecular, and epidemiological characterization of the SARS-CoV-2 virus and the Coronavirus Disease 2019 (COVID-19), a comprehensive literature review. *Diagn Microbiol Infect Dis* 2020; **98**(1):115094.
9. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. *BMJ* 2020; **370**: 3320.
10. Dubey P, Reddy SY, Manuel S, Dwivedi AK. Maternal and neonatal characteristics and outcomes among COVID-19 infected women: an updated systematic review and meta-analysis. *Eur J Obstet Gynecol Reprod Biol* 2020; **252**: 490-501

11. Molloy EJ, Lavizzari A, Klingenberg C, Profit J, Zupancic J, Davis A et al. Neonates in the COVID-19 pandemic. *Pediatric research* 2020 Aug.
12. Vivanti AJ, Vauloup-Fellous C, Prevot S, Zupan V, Suffee C, Cao J et al. Transplacental transmission of SARS-CoV-2 infection. *Nat Commun* 2020 ; **11**: 3572.
13. Fenizia C, Biasin M, Cetin I et al. Analysis of SARS-CoV-2 vertical transmission during pregnancy. *Nat Commun* 2020; **11**: 5128.
14. Correia CR, Marçal M, Vieira F, Santos E, Novais C, Maria AT et al. Congenital SARS-CoV-2 Infection in a Neonate With Severe Acute Respiratory Syndrome. *Pediatr Infect Dis J* 2020; **39**(12): e439-43.
15. Algarroba GN, Hanna N, Rekawek P, Vahanian S, Khullar P, Palaia T et al. Confirmatory evidence of the visualization of severe acute respiratory syndrome coronavirus 2 invading the human placenta using electron microscopy. *Am J Obstet Gynecol* 2020; **223**(6):953-4.
16. Hosier H, Farhadian S, Morotti R, Deshmukh U, Lu-Culligan A, Campbell K et al. SARS-CoV-2 infection of the placenta. *The Journal of Clin Invest* 2020; **130**(9): 4947-53.
17. Patane L, Morotti D, Giunta M, Sigismondi C, Piccoli M, Frigerio L et al. Vertical transmission of coronavirus disease 2019: severe acute respiratory syndrome coronavirus 2 RNA on the fetal side of the placenta in pregnancies with coronavirus disease 2019-positive mothers and neonates at birth. *Am J Obstet Gynecol* 2020; **2**(3): 100145.
18. Kreis NN, Ritter A, Louwen F, Yuan J. A Message from the Human Placenta: Structural and Immunomodulatory Defense against SARS-CoV-2. *Cells* 2020; **9**(8): 1777.
19. Ashary N, Bhide A, Chakraborty P, Colaco S, Mishra A, Chhabria K et al. Single-cell RNA-seq identifies cell subsets in human placenta that highly expresses factors driving pathogenesis of SARS-CoV-2. *Front Cell Dev Biol* 2020; **8**: 783.
20. Pique-Regi R, Romero R, Tarca AL, Luca F, Xu Y, Alazizi A et al. Does the human placenta express the canonical cell entry mediators for SARS-CoV-2? *Elife 9* 2020; **9**: e58716.
21. Moore KM, Suthar MS. Comprehensive analysis of COVID-19 during pregnancy. *Biochem Biophys Res Commun* 2020; **20**: 32241-5.
22. Lye P, Dunk C, Zhang J, Wei Y, Nakpu J, Hamada H et al. SARS-CoV-2 cell entry gene ACE2 expression in immune cells that infiltrate the placenta in infection-associated preterm birth. *MedRxiv* 2020; 20201590.

23. Facchetti F, Bugatti M, Drera E, Tripodo C, Sartori E, Cancila V et al. SARS-CoV-2 vertical transmission with adverse effects on the newborn revealed through integrated immunohistochemical, electron microscopy and molecular analyses of Placenta. *EBioMedicine* 2020; **59**: 102951.
24. Delorme-Axford E, Sadovsky Y, Coyne CB. The Placenta as a Barrier to Viral Infections. *Annual Review of Virology* 2014; **1**(1), 133–46.
25. 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.
26. Zaigham M, Andersson O. Maternal and perinatal outcomes with COVID-19: A systematic review of 108 pregnancies. *Acta Obstet Gynecol Scand* 2020; **99**: 823-9.
27. Ronnje L, Länsberg JK, Vikhareva O, Hansson SR, Herbst A, Zaigham M. Complicated COVID-19 in pregnancy: a case report with severe liver and coagulation dysfunction promptly improved by delivery. *BMC Pregnancy Childbirth* 2020; **20**(1): 511.
28. Kolkova Z, Bjurström M, Länsberg JK, Svedas E, Hamer M, Hansson S et al. Obstetric and intensive-care strategies in a high-risk pregnancy with critical respiratory failure due to COVID-19: A case report. *Case Rep Womens Health* 2020; **27**: e00240.
29. Henderson LA, Canna S, Schulert G, Volpi S, Lee P, Kernan K, Caricchio R et al. On the alert for cytokine storm: Immunopathology in COVID-19. *Arthritis Rheumatol* 2020; **72**(7): 1059-63.
30. Schoenmakers S, Snijder P, Verdijk R, Kuiken T, Kamphuis S, Koopman L et al. SARS-CoV-2 placental infection and inflammation leading to fetal distress and neonatal multi-organ failure in an asymptomatic woman. *medRxiv* 2020.06.08.20110437. Available at: https://doi.org/10.1101/20020.06.08.20110437
31. WF Ng, Wong SF, Lam A, Mak YF, Lee KC, Chow KM et al. The placentas of patients with severe acute respiratory syndrome: a pathophysiological evaluation. *Pathology* 2006; **38**(3): 210-8.
32. Wiberg N, Källen K, Herbst A, Åberg A, Olofsson P. Lactate concentration in umbilical cord blood is gestational age-dependent: a population-based study of 17 867 newborns. *BJOG* 2008; **115**(6): 704-9.
33. Kohler PF, Farr RS. Elevation of cord over maternal IgG immunoglobulin: evidence for an active placental IgG transport. *Nature* 1966; **210**(5040): 1070-1.

34. Zeng H, Xu C, Fan J, Tang Y, Deng Q, Zhang W et al. Antibodies in Infants Born to Mothers With COVID-19 Pneumonia. *JAMA* 2020; **323**(18): 1848-9.
35. Park D, Huh H, Kim Y, Son D, Jeon H, Im E et al. Analysis of intrapatient heterogeneity uncovers the microevolution of Middle East respiratory syndrome coronavirus. *Cold Spring Harb Mol Case Stud* 2016; **2**(6): a001214.
36. Jary A, Leducq V, Malet I, Marot S, Klement-Frutos E, Tessyou E et al. Evolution of viral quasispecies during SARS-CoV-2 infection. *Clin Microbiol Infect* 2020; **26**(11):1560e1-4.
37. Rambaut A. Phylodynamic Analysis.Virological. Published 6 march 2020. Accessed 5 january 2020. Available at: http://virological.org/t/phylodynamic-analysis-176-genomes-6-mar-2020/356
38. Su YC, Anderson DE, Young be, Zhu F, Linster M, Kalimuddin S et al. Discovery of a 382-nt deletion during the early evolution of SARS-CoV-2. *BioRxiv* 2020; doi:10.1101/2020.03.11.987222.

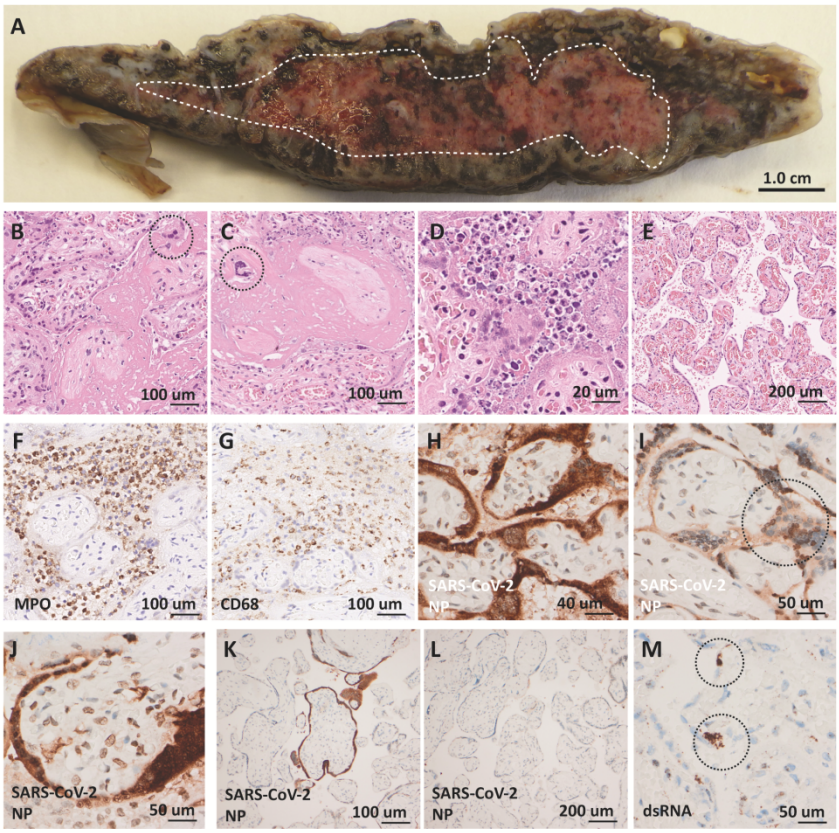

Figure 2.
Placental pathology. (A) Transected placenta with confluent accumulation of fibrinoid demarcated (white broken line). (B-C) Massive intervillous fibrinoid deposition surrounding denuded villi with extravillous syncytiotrophoblasts (circles) located in the fibrinoid. (D) Acute intervillositis with karrhyorectic neutrophils in the intervillous space and degeneration of the villous trophoblast layer. (E) Representative region of chorangiosis. (F-G) Immunohistochemical staining for myeloperoxidase (MPO) and CD68 with positivity in inflammatory cells in areas of intervillositis. (H-J) Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) nucleoprotein (NP) detected in nucleus and (circle in I) and cytoplasm in villous trophoblasts and syncytiotrophoblasts as well as in the nucleus of villous stromal cells. (J) in areas of intervillositis. (K-L) Areas without intervillositis showed absent or focal staining for SARS-CoV-2 nucleoprotein of villi. (M) Double stranded RNA (dsRNA) detected in villous trophoblasts and syncytiotrophoblasts (circles).

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