Vertical Transmission of SARS-CoV-2 is Plausible During Pregnancy and Vaginal Delivery: A Prospective Cohort Study

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Abstract

Objective: To evaluate if SARS-CoV-2 is detectable in vaginal swabs and whether antibodies against SARS-CoV-2 are present in maternal and umbilical cord blood of pregnant women with COVID-19. Design: Prospective cohort study. Setting: Department of Obstetrics and Gynaecology, Copenhagen University Hospital – North Zealand, Denmark. Population: Pregnant women tested positive for SARS-CoV-2 in a pharyngeal swab between August 20th, 2020 and March 1st, 2021 who gave birth during the same period. Methods: Maternal blood sample and vaginal swabs were collected at inclusion. If included during pregnancy, these samples were repeated at delivery in addition to an umbilical cord blood sample. Swabs were analysed for SARS-CoV-2 and blood samples for SARS-CoV-2 total antibodies. Placental and neonatal swabs were performed on clinical indications. Main outcome measures: SARS-CoV-2 in vaginal swabs and SARS-CoV-2 total antibodies in maternal and umbilical cord blood. Results: We included 28 women, hereof 4 serious maternal or fetal outcomes including 1 neonatal death. Within the first eight days after a maternal positive pharyngeal swab, SARS-CoV-2 was detectable in two vaginal (2/28) and two placental swabs (2/4), whereas SARS-CoV-2 antibodies were detected in 1/13 women. After eight days, SARS-CoV-2 was not detectable in vaginal swabs and SARS-CoV-2 antibodies were observed in 19/21 of women. Antibodies in cord blood of seropositive mothers appeared after 16 days. Conclusion: Vertical transmission of SARS-CoV-2 seems plausible since SARS-CoV-2 is detectable in the vagina up to eight days after a positive pharyngeal swab at which time the neonate is not yet protected by antibodies.

Vertical Transmission of SARS-CoV-2 is Plausible During Pregnancy and Vaginal Delivery: A Prospective Cohort Study

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ABSTRACT

Objective: To evaluate if SARS-CoV-2 is detectable in vaginal swabs and whether antibodies against SARS-CoV-2 are present in maternal and umbilical cord blood of pregnant women with COVID-19.

Design: Prospective cohort study.

Setting: Department of Obstetrics and Gynaecology, Copenhagen University Hospital – North Zealand, Denmark.

Population: Pregnant women tested positive for SARS-CoV-2 in a pharyngeal swab between August 20th, 2020 and March 1st, 2021 who gave birth during the same period.

Methods: Maternal blood sample and vaginal swabs were collected at inclusion. If included during pregnancy, these samples were repeated at delivery in addition to an umbilical cord blood sample. Swabs were analysed for SARS-CoV-2 and blood samples for SARS-CoV-2 total antibodies. Placental and neonatal swabs were performed on clinical indications.

Main outcome measures: SARS-CoV-2 in vaginal swabs and SARS-CoV-2 total antibodies in maternal and umbilical cord blood.

Results: We included 28 women, hereof 4 serious maternal or fetal outcomes including 1 neonatal death. Within the first eight days after a maternal positive pharyngeal swab, SARS-CoV-2 was detectable in two vaginal (2/28) and two placental swabs (2/4), whereas SARS-CoV-2 antibodies were detected in 1/13 women. After eight days, SARS-CoV-2 was not detectable in vaginal swabs and SARS-CoV-2 antibodies were observed in 19/21 of women. Antibodies in cord blood of seropositive mothers appeared after 16 days.

Conclusion: Vertical transmission of SARS-CoV-2 seems plausible since SARS-CoV-2 is detectable in the vagina up to eight days after a positive pharyngeal swab at which time the neonate is not yet protected by antibodies.

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Keywords : Severe acute respiratory syndrome coronavirus 2; COVID-19; Obstetric delivery; Pregnancy complications; Pregnancy outcome; Placental dysfunction; Vertical transmission; Cohort studies; Prospective studies

Tweetable abstract: Vertical transmission of SARS-CoV-2 is plausible within the first week of maternal COVID-19.

ABBREVIATIONS

BMI: Body Mass Index

CTG: cardiotocography

COVID-19: Coronavirus disease 2019

NICU: Neonatal Intensive Care Unit

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

INTRODUCTION

Whether vertical transmission of SARS-CoV-2 from mother to child may occur during maternal infection in pregnancy is still unclear (1, 2). Possible mechanisms of prenatal transmission between mother and child include transplacental transmission and ascending infection from the vagina through the cervix. Intrapartum and postpartum transmission might happen through fetal ingestion or aspiration of vaginal and fecal secretions during vaginal delivery, through respiratory droplets, or breast feeding (1). Few studies have detected SARS-CoV-2 in vaginal secretions (3-6). A recent case report from Sweden found evidence of transplacental vertical transmission of SARS-CoV-2 in gestational week 35 (7).

The presence of antibodies in fetal blood might offer protection of the fetus and child against COVID-19 during pregnancy, vaginal delivery, and in the neonatal period. SARS-CoV-2 total antibodies have been detected in less than 40% of non-pregnant individuals within the first week after infection (8). One study showed that the SARS-CoV-2 antibody titre remained stable from infection in the first trimester until delivery, but the exact timing of maternal seroconversion was not investigated (9). Other studies have examined the presence of SARS-CoV-2 antibodies at delivery in blood samples from the umbilical cord or the offspring of mothers with COVID-19. Some found anti-SARS-CoV-2 IgG, others both IgM and IgG (3, 10, 11). Since IgG is the only antibody class that significantly crosses the placenta, findings of IgM in cord blood indicates in utero infection, while IgM in neonates could be caused by in utero infection as well as postnatal infection. Additionally, the timespan between maternal SARS-CoV-2 diagnosis and the presence of antibodies in umbilical cord has been assessed, however, findings have been ambiguous (3). Together, previous studies may indicate that the risk of vertical transmission is increased if the woman delivers less than one week from onset of infection.

The objective of this study was to investigate if SARS-CoV-2 is detectable in vaginal swabs of pregnant women diagnosed with COVID-19 and to study the presence and timing of SARS-CoV-2 antibodies in maternal and umbilical cord blood during and after COVID-19 in pregnancy.

METHOD

During the second wave of the COVID-19 pandemic in Denmark, we conducted a prospective cohort study entitled the "CareMum COVID-19 study" from August 20th, 2020 to March 1st, 2021, at the Department of Obstetrics and Gynaecology, Copenhagen University Hospital – North Zealand with approximately 4000 annual deliveries.

We included pregnant women who tested positive for SARS-CoV-2 in a pharyngeal swab at a test centre or during a routine test when entering the antenatal clinic or labour ward. Women were eligible if they were to deliver within the study period and were able to give written and oral informed consent in English or Danish. Inclusion took place either during pregnancy at the antenatal clinic or when women were admitted for delivery. All participants had vaginal swabs and maternal blood samples done at the time of inclusion. If included during pregnancy, the samples were repeated at delivery where an umbilical cord blood sample was also taken. At the time of delivery, two vaginal swabs were done – the first, during the initial vaginal examination, and the second, during the active phase of delivery or immediately after. Vaginal swabs were frozen at -20°C until analysis (0-30 days, median 5 days). A maternal blood sample was taken just before or immediately after delivery, and an umbilical cord blood sample was performed immediately after birth. Blood samples were frozen at -80°C until analysis.

In some cases, swabs from the neonate and the placenta, as well as a placental histopathological examination and one fetal autopsy, was performed on clinical indications. Placental swabs were done from both the maternal and the fetal side, and neonatal swabs from the axillary fold, naso-, and oropharynx. These samples were not part of the study protocol, but results are described when present.

Pharyngeal swabs as well as vaginal and placental swabs were analysed by RT-PCR as part of the routine diagnostics (see supporting information, appendix S1, for further information).

Total SARS-CoV-2 antibodies in maternal and cord blood samples were analysed using a qualitative (reactive/non-reactive) biochemical assay, The VITROS Immunodiagnostic Product Anti-SARS-CoV-2 Total (CoV2T), developed by Ortho-Clinical Diagnostics. The test result (S/C) = (signal for test sample / signal at cutoff value) [?] 1.00 is considered reactive (i.e. positive for antibodies) and result < 1.00 is considered non-reactive (i.e. negative for antibodies).

We obtained demographic and clinical data of participants as well as non-participants in the "CareMum COVID-19 study" from the Danish "COVID-19 in pregnancy" database, which contains information based on medical records on all women diagnosed with COVID-19 during pregnancy in Denmark as described elsewhere (12). Non-participants were women who tested positive for SARS-CoV-2 and gave birth within the study period but who for various reasons were not included in this study (e.g. women did not understand and read Danish/English, did not want to participate or were not asked due to busyness at the maternity ward). Case completeness was secured by a retrospective registry linkage to national databases covering information on results from SARS-CoV-2 pharyngeal swabs. Furthermore, participants were asked to complete a questionnaire about COVID-19 symptoms at the time of inclusion. Symptoms of non-participants were not systematically reported.

Data was analysed using SPSS version 27 (SPSS Inc., Chicago, IL). Categorical variables are presented as number with percentage and continuous variables as mean with standard deviation (SD) or median with interquartile range as appropriate. Analyses of differences between means, medians and proportions were performed using Student's T-test, Mann-Whitney, or Fischer's Exact test, respectively. The study was approved by The Regional Committee on Health Research Ethics.

RESULTS

We included 28 of 36 (77.8%) women, who had SARS-CoV-2 positive pharyngeal swabs during pregnancy in the study period. Three participants were not tested by RT-PCR. Participant 6 was tested by a nasopharyngeal antigen-test, and participants 16 and 18 were tested at a private test centre. Participants and non-participants did not differ regarding baseline characteristics (table 1). Twenty-two participants (78.6%) were included at delivery (table 2), while six participants were included and examined during pregnancy with examinations repeated at delivery (table 3). Participants tested positive for SARS-CoV-2 by a pharyngeal swab between gestational age (GA) 25 weeks (w) and 0 days (d) to 41w4d, and the onset of symptoms was reported from 16 days before and until three days after the positive swab.

Vaginal swabs were obtained from all participants, and SARS-CoV-2 was detected in 2 of 28 cases – at

one and eight days, respectively, after the positive pharyngeal swab (7.1%). One vaginal swab test was inconclusive. No positive vaginal swabs were found during vaginal delivery, independently of the timing with onset of symptoms or a positive pharyngeal swab (table 2 and 3).

Maternal antibodies were analysed in 28 women (table 2 and 3). Within 8 days of a positive pharyngeal swab, SARS-CoV-2 antibodies were detected in only 1 (participant 6, table 2) of 13 women (7.7%). Whereas, antibodies were observed in 19 of 21 (90.5%) women who had a positive pharyngeal swab more than 8 days prior to the antibody test (table 2 and 3). Two women had no antibody response in blood samples at day 57 and 74 days after infection, respectively (participants 25 and 26).

Antibodies were analysed in 25 umbilical cord blood samples. Antibodies were not detected in umbilical cord blood from pregnancies where the woman delivered within 16 days of a SARS-CoV-2 positive pharyngeal swab (n=7). However, antibodies were present in 16 of 17 (94.1%) cord blood samples of pregnancies where the woman was seropositive and delivered more than 16 days after infection (table 2 and 3). The test results (S/C) of antibodies in maternal and cord blood at delivery were highly correlated (figure S3, R^2=0.64, p<0.00005). There was a positive correlation between the test results (S/C) for antibodies in cord blood and the number of days from SARS-CoV-2 positive pharyngeal swab to delivery (figure S4, R^2=0.43, p=0.002), whereas the correlation between the test result (S/C) for antibodies in maternal blood and the number of days between diagnosis and delivery was not statistically significant (figure S4, R^2=0.20, p=0.054). Three cord blood samples from children of nineteen seropositive mothers were without detectable antibodies (15.8%, participants 6, 8 and 10).

Samples performed on clinical indication (table S1)

Placental swabs

Placental swabs were done on clinical indication in four cases (Table 2 and S1, participants 3, 4, 5 and 6). SARS-CoV-2 was detected in two cases (participants 5 and 6), both of which had adverse fetal outcomes (described in details below).

Placental pathology

Two placental histopathological examinations and one fetal autopsy were performed which are described below (participants 5 and 6).

Neonatal swabs and neonatal COVID-19

Swabs from the neonates were done in two cases (participants 4 and 6). Neither were positive for SARS-CoV-2 and overall, no children were diagnosed with COVID-19 after birth.

Clinical outcomes and complications in pregnancies with COVID-19

Participant 5 was diagnosed with SARS-CoV-2 at GA 29w1d. She presented at the antenatal ward at GA 29w6d with reduced fetal movements and vaginal bleeding. The neonate was delivered the same day by emergency caesarean section due to a pathological cardiotocography (CTG) and was born small for gestational age (birth weight 1235 g, Z-score -1.87). APGAR was 8 after 5 minutes and umbilical cord blood with respiratory compensated metabolic acidosis (arterial pH 7.23, Base Excess -10.4 and venous pH 7.33, Base Excess -12.0). Microscopic examination of the placenta showed severe acute and chronic intervillositis with necrosis of the trophoblast (Figure S1). Swabs from the fetal side of the placenta were positive for SARS-CoV-2. The results of the vaginal swabs were inconclusive. The neonate was admitted to NICU for 40 days. The mother had a complicated puerperal period with fever, fatigue, and diarrhea. Yersinia enterocolitica was found in a maternal fecal sample. Bacterial culture from the placenta was negative. SARS-CoV-2 antibodies were not detected in either maternal or in cord blood. Eleven weeks after delivery, anti-cardiolipin antibodies were found associated with an increased risk of arterial and venous thrombosis. The findings could be consistent with intrauterine SARS-CoV-2 infection, but bacterial infection could not be ruled out.

Participant 6 tested positive for SARS-CoV-2 at GA 31w2d. Her partner was tested positive the same day. She presented at the antenatal ward at 32w0d with a reduction in fetal movements but was discharged after a normal CTG. At 32w3d, she presented with contractions. The neonate was delivered shortly after by emergency caesarean section due to a preterminal CTG with APGAR 0 after 5 minutes and arterial pH 6.82, Base Excess -17.5 and venous pH 6.85, Base Excess -17.2. Birth weight was 2000 g and Z-score -0.29. One hour after delivery, the neonate was declared dead after a prolonged neonatal resuscitation attempt. Autopsy of the neonate showed no malformations, but signs of asphyxia from petechiae and meconium aspiration. In addition, there was some histologic evidence of acute thymic involution and adrenal stress-related changes, but no signs of prolonged intrauterine stress or growth restriction. Microscopic examination of the placenta showed severe abnormality with acute and chronic intervillositis and abundant perivillous fibrin deposits comprising 70% of the parenchyma (Figure S2). SARS-CoV-2 was detected in the vaginal swab at delivery and in swabs from both the fetal and maternal side of the placenta. Neonatal swabs were SARS-CoV-2 negative. The mother tested positive for SARS-CoV-2 antibodies while umbilical cord blood tested negative. Blood and urine culture at the time of delivery were negative. The placental findings, potentially linked to intrauterine SARS-CoV-2 infection were likely to have caused the fatal fetal outcome.

Participant 27 was diagnosed with gestational diabetes mellitus at GA 13w0d. She tested positive for SARS-CoV-2 at GA 27w2d and was hospitalized at GA 28w0d due to COVID-19. During the admission, she was diagnosed with three segmental lung embolisms and a secondary pneumonia was suspected. She was treated with Low Molecular Weight Heparins, Remdesivir, antibiotics, and prednisolone. Vaginal swabs analysed for SARS-CoV-2 were negative. She gave birth by an uncomplicated planned caesarean section at GA 38w0d. During admission, no antibodies were detected in maternal blood eight days after the SARS-CoV-2 positive pharyngeal swab, whereas at delivery, 75 days after the initial infection, SARS-CoV-2 antibodies were detectable in both maternal and cord blood samples.

Participant 28 presented at the antenatal ward at GA 24w5d with vaginal bleeding and at GA 25w1d with symptoms of deep vein thrombosis of the femoral vein, which was confirmed by ultrasound. She started treatment with Low Molecular Weight Heparin. The vaginal bleeding was derived from a varicose vein in the left labium minor. Eleven days later (GA 26w5d), she contacted the Emergency Department due to shortness of breath and vomiting. There was no suspicion of pulmonary embolism or severe COVID-19. She tested positive for SARS-CoV-2 by a pharyngeal swab and was admitted for two days. At day one after the positive pharyngeal swab, two vaginal swabs were positive for SARS-CoV-2, but no maternal antibodies were detected. Blood culture and urine culture taken after admission to the hospital with COVID-19 were negative. She gave birth by an uncomplicated planned caesarean section at GA 38w5d. On day 16 and 37 after the positive swab and at delivery (day 84), SARS-CoV-2 antibodies were detected in maternal blood samples. At delivery, antibodies were also detected in cord blood.

Besides the abovementioned four severe cases, a few other complications were found among participants during pregnancy and delivery (table 1).

DISCUSSION

Main Findings

SARS-CoV-2 was detected in vaginal swabs of two pregnant women with COVID-19 and in placental swabs of two severe fetal cases after maternal COVID-19. Within the first eight days of a positive pharyngeal swab, SARS-CoV-2 antibodies were detected in only 7.7% of the women, while SARS-CoV-2 antibodies were observed in 90.5% women tested more than eight days after a positive swab. Antibodies in umbilical cord blood were not detected within the first 16 days after the mother was tested positive, hereafter, antibodies were present in 94.1% of cord blood samples of seropositive mothers. We observed two severe fetal outcomes both associated with maternal admittance due to reduced fetal movements, pathological or preterminal CTG, delivery by emergency preterm caesarean section, and abnormal placental histopathological examinations.

Strengths and limitations

The prospective design is a strength of our study. Participants were compared with all non-participants by a retrospective registry linkage, enabling us to examine whether participants were representative of all pregnant women with COVID-19 at the index hospital during the study period. Additionally, tests for SARS-CoV-2 in Denmark are free of charge and performed at public test centres reporting the results to national databases, which reduced the number of undetected cases of COVID-19. Finally, we used a serological assay with a high diagnostic accuracy with a sensitivity of 95.3% and a specificity of 100% (13).

The study has several limitations. We included 77.8% of eligible women. However, participants and non-participants did not differ with regards to background characteristics. We had no samples from mothers or cord blood between eight and 16 days after a positive maternal pharyngeal swab, entailing the exact timing of seroconversion among pregnant women with COVID-19 unknown. Further, most vaginal swabs were kept at -20*C until analysis, possibly causing a deterioration of SARS-CoV-2 during storage, decreasing our ability to detect SARS-CoV-2 in the vaginal samples. However, a previous study found that SARS-CoV-2 RNA was stable when stored at -20degC for up to 84 days (14). Lack of infection status from the participants' partners is a limitation, since it is not fully evaluated whether SARS-CoV-2 can be transmitted through semen and cause detection of SARS-CoV-2 in the vagina (15). We measured total antibodies and did not discriminate between IgG and IgM. Finally, three participants were not tested by pharyngeal RT-PCR (one by an antigen-test and two at private test centres), thus exact details about analysis methods are lacking. However, the participants were positive for SARS-CoV-2 antibodies.

Interpretation

Other studies have examined whether SARS-CoV-2 is detectable in vaginal swabs. In contrast to our study, the majority of studies report negative vaginal swabs for SARS-CoV-2 (1, 16-19). However, there are reports of SARS-CoV-2 detected in vaginal swabs of both pregnant (n=3), reproductive-aged (n=1), and post-menopausal (n=1) women, indicating that transmission during vaginal delivery might be possible, although the risk is likely to be low (3-6). In accordance with our findings, other studies have also found positive placental swabs, indicating a viral spread that is potentially either an ascending infection or infection through the bloodstream (n=5) (3, 4, 6, 20). Other studies have demonstrated SARS-CoV-2 in neonatal swabs of children born to women with symptoms of COVID-19 (16, 17, 21, 22), which we did not find.

There are several potential sources for viral presence in the vagina. The coronavirus binds to target cells through angiotensin-converting enzyme 2 (ACE2) receptors, which are upregulated in vaginal epithelium during pregnancy, making it possible for the virus to bind (23). Alternatively, it is possible that SARS-CoV-2 is detected because of exudation from the bloodstream rather than release from the epithelium itself. Detection of SARS-CoV-2 in the vagina could also be due to fecal or seminal contamination (15, 24).

Our study indicates that the neonate is not protected in the acute phase of maternal COVID-19 (between 0-16 days after a positive maternal pharyngeal swab) as there were no SARS-CoV-2 antibodies in cord blood even though the mother had produced antibodies. However, after 16 days 94.1% of offspring had antibodies in cord blood. Other studies have assessed the timespan between maternal SARS-CoV-2 diagnosis and the presence of antibodies in cord blood, and a study found IgG in 11 cord blood samples and both IgG and IgM in one case of 31 cases in total. In one case IgG was found as early as one day after maternal SARS-CoV-2 infection (3). However, IgG is usually not detected before 1-2 weeks after acute viral infection and the mother may therefore have been infected several days before diagnosis. Another study found that 11 infants of 83 seropositive mothers (13%) did not have antibodies in cord blood after a median time of six days (interquartile range 0-12 days) from diagnosis to delivery (25). This time span is shorter than we report with a range of 0-16 days. In accordance with our study, they found a positive correlation between the levels of maternal and cord-blood IgG (25).

The adverse fetal outcomes of participant 5 and 6 in our study are similar to a confirmed case of transplacental transmission of SARS-CoV-2 from Sweden, where a pregnant woman with a three-day history of COVID-19 presented with reduced fetal movements at GA 34w4d (7). After an emergency caesarean section due to a pathological CTG the mother and child were separated. Placental pathology was similar to the findings in our

study as well as other studies (4, 7, 26-28). Authors concluded that the neonate had suffered from transient asphyxia attributed to intrauterine hypoxia secondary to placental dysfunction. The case from Sweden (7) and others (4), as well as participants 5 and 6 in our study, suggests that reduced fetal movements during COVID-19 should be handled with aggravated concern.

In our study, two cases did not have antibodies in neither maternal nor umbilical cord blood (participants 25 and 26). Participant 25 had a low CT value, but a negative pharyngeal swab four days later, while participant 26 had no symptoms, a high CT value, and a negative pharyngeal swab the day after testing positive. Possible explanations could be either a false positive pharyngeal swab, a false negative antibody tests or that the women did not have an antibody response. In three other cases, antibodies were not detected in cord blood of seropositive mothers, which in two of the cases was possibly due to a short timespan from SARS-CoV-2 infection to delivery (8 and 16 days, respectively).

In large cohort studies, the overall risk of severe neonatal outcomes as well as neonatal infection related to maternal COVID-19 seems low (29). However, our findings indicate that vaginal testing for SARS-CoV-2 in women with COVID-19 within eight days before going into labour could be considered. In case of a positive vaginal swab, mode of delivery should be discussed with the woman as passive immunity of the neonate is not guaranteed.

CONCLUSION

Vertical transmission of SARS-CoV-2 during vaginal delivery seems rare, but is plausible within the first week of infection, since SARS-CoV-2 is detectable in the vagina and antibodies do not yet protect the neonate at this time. Further, maternal COVID-19 may in rare cases cause intrauterine infection and fetal asphyxia due to placental dysfunction. We propose that reduced fetal movements in pregnancies affected by COVID-19 should be handled with aggravated concern.

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Disclosure of Interests

None of the authors have any conflicts of interest to declare.

Contribution to Authorship

TDC, PBA, JMB, and EL designed the study. TDC applied for the ethical approval. JM and VH recruited the participants and collected the samples. LR was responsible for analysis of anti-SARS-CoV-2 in blood samples. CJ was responsible for the initial handling of blood samples. AA was responsible for providing baseline data on participants as well as non-participants. TEO performed the pathological examination. LN and MF were responsible for analysis of swabs for SARS-CoV-2. JM and VH wrote the first draft of the manuscript with subsequent amendments by all authors, who also approved the final version.

Details of Ethics Approval

The Regional Committee on Health Research Ethics in the capital region of Denmark approved the study on May 3rd, 2020 with an additional protocol approved November 17th, 2020 (H-20028002). Written informed consent was collected from all participants before inclusion in the study. Furthermore, a written informed consent for publication was obtained from participant 5, 6, 27, and 28. Demographic and clinical data of participants as well as non-participants were extracted from the prospective national observational study "COVID-19 in pregnancy", which was approved by the Danish Patient Safety Authority on April 24th, 2020 (reg. no. 31-1521-252) and the regional Data Protection Agency in Region Zealand on March 23rd, 2020 (reg. no. REG022-2020).

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TABLE CAPTION LIST

Table 1 : Baseline characteristics (demographic and clinical presentation)

Table 2 : Pregnant women tested positive for SARS-CoV-2 during pregnancy or delivery - data sampled at delivery only

Table 3 : Pregnant women tested positive for SARS-CoV-2 during pregnancy - data sampled at diagnosis as well as at delivery

SUPPORTING INFORMATION

Appendix S1: Method description regarding RT-PCR analysis of swabs for SARS-CoV-2

Figure S1 : Placental pathology, participant 5. (A) Transected placenta, cut surface with patchy white fibrin deposit. (B) Acute and chronic intervillositis with necrosis of the trophoblast layer (black arrow) accompanied by fibrin deposits (white arrow). H&E.

Figure S2 : Placental pathology, participant 6. (A) Transected placenta, cut surface with multiple white areas of confluent fibrin deposits (white arrows). (B) Acute and chronic intervillositis (black arrow) accompanied by abundant fibrin deposits (white arrow). H&E.

Figure S3: Correlation between maternal and umbilical cord blood test result (S/C) for total antibodies. Mothers non-reactive for antibodies are excluded. Mother-infant pairs, where the umbilical cord blood sample was missing, are excluded.

Figure S4: Test result (S/C) for total antibodies in maternal and umbilical cord blood and the number of days between diagnosis and delivery. Mothers non-reactive for antibodies are excluded. Mother-infant pairs, where the umbilical cord blood sample was missing, are excluded.

Table S1: Samples performed at delivery on clinical indications

Table 1: Baseline characteristics (demographic and clinical presentation)

	Participants (n=28)	**Non-participants (n=8)**
Maternal age (years)	30.0 (±4.5)	30.0 (±7.9)
Pre-pregnancy BMI (kg/m^2)	24.25 (20.8;28.2)	27.93 (20.5;30.1)
Current smoker	2 (7.1)	0

	Participants (n=28)	**Non-participants (n=8)**
Parity, nulliparous	11 (39.3)	3 (37.5)
Ethnicity, Caucasian	15 (53.6)	4 (50.0)
Pre-existing medical disorders*	7 (25.0)	2 (25.0)
Presenting symptoms	**Presenting symptoms**	**Presenting symptoms**
Headache	18 (64.3)	
Cough	19 (67.9)	
Sore throat	16 (57.1)	
Nasal congestion	18 (64.3)	
Muscle soreness	11 (39.3)	
Fever	16 (57.1)	
Anosmia	13 (46.4)	
Dyspnoea	7 (25.0)	
Diarrhoea	4 (14.3)	
Asymptomatic	4 (14.3)	
Maternal admission due to COVID-19	**Maternal admission due to COVID-19**	**Maternal admission due to**
Medical ward	3 (10.7)	0
ICU	0	0
Pregnancy complications	**Pregnancy complications**	**Pregnancy complications**
Gestational hypertension	1 (3.6)	0
Preeclampsia	0	1 (12.5)
Deep venous thrombosis	1 (3.6)	0
Pulmonary embolism	1 (3.6)	0
Gestational diabetes mellitus	2 (7.1)	1 (12.5)
Delivery mode	**Delivery mode**	**Delivery mode**
Vaginal delivery	19 (67.9)	4 (50.0)
Vaginal, vacuum extraction	1 (3.6)	0
Elective caesarean section	4 (14.3)	1 (12.5)
Acute caesarean section	4 (14.3)	3 (37.5)
Perinatal outcomes	**Perinatal outcomes**	**Perinatal outcomes**
GA (days)	280.0 (269.8;285.8)	269.5 (259.6;280.0)
Preterm delivery (< 37+0 weeks)	2 (7.1)	1 (12.5)
Offspring gender (girl)	16 (57.1)	4 (50.0)
Birth weight (Z-score)	-0.10 (-0.8;0.5)	-0.73 (-1.7;0.0)
Artery pH < 7.1	1 (4.5)	0
Apgar (5 min) < 7	1 (3.6)	0
NICU	3 (10.7)	2 (25.0)
Neonatal death	1 (3.6)	0

*Essential hypertension (n=1), Factor V Leiden mutation (n=1), beta-thalassemia (n=1), hypothyroidism (n=3), PCOS (n=1), depression (n=2).

Data presented as means (±SD), medians (25;75% percentiles) or proportions (%). For some of the variables, proportions are changing because of missing data. Differences between means, medians and proportions were analysed by Student's T-test, Mann-Whitney or Fischer's Exact test, respectively. BMI: Body Mass Index. ICU: Intensive Care Unit. GA: gestational age. NICU: Neonatal Intensive Care Unit.

Table 2: Pregnant women tested positive for SARS-CoV-2 during pregnancy or delivery - data sampled at delivery only

Pt	**Data sampled at delivery**	**Data sampled at delivery**

	Days between SARS-CoV-2 positive pharyngeal swab and delivery	SARS-CoV-2 positive pharyngeal
1	0	38w1d
2	1	37w5d
3	1	41w0d
4	1	41w4d
5	5	29w1d
6	8	31w2d
7	8	39w3d
8	16	38w6d
9	30	35w5d
10	30	36w5d
11	35	35w3d
12	38	31w4d
13	51	34w5d
14	61	31w4d
15	63	30w0d
16	64	30w5d
17	66	30w4d
18	69	30w6d
19	79	28w5d
20	86	26w0d
21	91	26w6d
22	102	25w0d

Abbreviations: CTG: cardiotocography; CS: cesarean section; GA: gestational age; w: weeks; d: days; VD: vaginal delivery; E-CS: emergency cesarean section; Pt: Participant; SARS-CoV-2: Severe acute respiratory coronavirus-2; SGA: small for gestational age; N/A: not available. Results of swabs analysed for SARS-CoV-2 are shown as "+" for a positive result and "-" for a negative result. Test result (S/C) = (signal for test sample / signal at cutoff value) [?] 1.00 is considered reactive (i.e. positive for antibodies) and result < 1.00 is considered non-reactive (i.e. negative for antibodies). * = If emergency cesarean section, then two vaginal swabs were performed at an initial vaginal examination and immediately before/after the emergency CS respectively. If vaginal delivery then two vaginal swabs were performed, one before an initial vaginal examination and one during the active phase of delivery or immediately after, respectively. ** = Vaginal swabs performed 1 day postpartum. *** = only one vaginal swab was performed due to E-CS.

Table 3: Pregnant women tested positive for SARS-CoV-2 during pregnancy - data sampled at diagnosis as well as at delivery

Pt	Data sampled during pregnancy	Data sampled during pregnancy	Data sampled during pregnancy	Data sampled during pregnancy	Data sampled during pregnancy	Data sampled at delivery	Data sampled at delivery	Data sampled at delivery	Data sampled at delivery	Data sampled at delivery

	Days between SARS-CoV-2 positive pharyngeal swab and sampled data	**SARS-CoV-2 positive pharyngeal swab (GA)**	**Onset of symptoms (GA)**	**Vaginal swabs for SARS-CoV-2***	**Maternal total antibodies (test result (S/C))**	**Days between SARS-CoV-2 positive pharyngeal swab and delivery**	**Delivery (GA)**	**Mode of delivery (VD/CS)**	**Vaginal swab for SARS-CoV-2****	**Total antibodies (Test result (S/C))**
										Maternal
23	1	37w1d	No symptoms	-/-	<1.00	26	40w6d	VD	-/-	7
24	5	35w6d	35w6d	-/-	<1.00	34	40w5d	VD	-/-	6
25	1	31w0d	28w4d	-/-	<1.00	57	39w1d	VD	-/-	<1.00
26	1	28w3d	No symptoms	-/-	<1.00	74	39w0d	VD	N/A	<1.00
27	8	27w2d	27w4d	-/-	<1.00	75	38w0d	P-CS	-/-	250
28	1	26wd5	26w4d	+/+	<1.00	84	38w5d	P-CS	-/-	484
	16			-/-	42					
	37			-/-	158					

Abbreviations: GA: gestational age; w: week; d: days; VD: vaginal delivery; P-CS: planned cesarean section; Pt: Participant; SARS-CoV-2: Severe acute respiratory coronavirus-2; N/A: not available. Results of swabs analysed for SARS-CoV-2 are shown as "+" for a positive result and "-" for a negative result. Test result (S/C) = (signal for test sample / signal at cutoff value) [?] 1.00 is considered reactive (i.e. positive for antibodies) and result < 1.00 is considered non-reactive (i.e. negative for antibodies). * = Two vaginal swabs performed at the same time. **= If planned cesarean section, the vaginal swabs were performed immediately before and after CS. If vaginal delivery, two vaginal swabs were performed, one before an initial vaginal examination and one during the active phase of delivery or immediately after, respectively.