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ORIGINAL RESEARCH ARTICLE

A prospective cohort study of confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during pregnancy evaluating SARS-CoV-2 antibodies in maternal and umbilical cord blood and SARS-CoV-2 in vaginal swabs

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Abstract

Introduction: Evidence about the consequences of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in pregnancy is rapidly increasing; however, data on antibody response and risk of transmission during pregnancy and delivery are still limited. The aim of this study was 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 confirmed SARS-CoV-2.

Material and methods: A single-unit prospective cohort study in Denmark including pregnant women with SARS-CoV-2 infection confirmed by a pharyngeal swab between August 20, 2020, and March 1, 2021, who gave birth during the same period. All patients admitted to the maternity ward and antepartum clinic were screened for SARS-CoV-2 infection. A maternal blood sample and vaginal swabs were collected at inclusion. If included antepartum, these samples were repeated intrapartum when an umbilical cord blood sample was also collected. Swabs were analyzed for SARS-CoV-2 and blood samples were analyzed for SARS-CoV-2 total antibodies. Placental and neonatal swabs as well as placental histopathological examinations were performed on clinical indications.

Results: We included 28 women, of whom four had serious maternal or fetal outcomes including one case of neonatal death. Within the first 8 days after confirmed

Abbreviations: COVID-19, coronavirus disease 2019; CTG, cardiotocography; IgG, immunoglobulin G; IgM, immunoglobulin M; RT-PCR, reverse transcription polymerase chain reaction; S/C, signal for test sample/signal at cut-off value; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Julie Milbak and Victoria MF Holten share first authorship.

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SARS-CoV-2 infection, SARS-CoV-2 was detectable in two vaginal swabs (2/28) and SARS-CoV-2 antibodies were detected in 1 of 13 women. From 16 days after confirmed infection, antibodies were observed in 19 of 21 of women. Antibodies in cord blood were not detected during the first 16 days after confirmed infection ($n = 7$). However, from 26 days, antibodies were present in 16 of 17 cord blood samples of seropositive mothers. Placental examination in two cases of severe fetal outcomes preceded by reduced fetal movements revealed SARS-CoV-2 in swabs and severe histopathological abnormalities.

Conclusions: SARS-CoV-2 was detected in only 2 of 28 vaginal swabs within 8 days after confirmed infection in pregnant women. Our data suggest that maternal seroconversion occurs between days 8 and 16, whereas antibodies in cord blood of seropositive mothers were present in the majority from 26 days after confirmed infection. Additional data are needed regarding timing of seroconversion for the mother and appearance of antibodies in cord blood.

KEYWORDS
cohort studies, coronavirus disease 2019, obstetric delivery, placental dysfunction, pregnancy complications, pregnancy outcome, prospective studies, severe acute respiratory syndrome coronavirus 2, vertical transmission

1 | INTRODUCTION

Whether transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from mother to child can 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 through breastfeeding.[1] Few studies have detected SARS-CoV-2 in vaginal secretions.[3–6] A recent case report from Sweden found evidence of transplacental 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 coronavirus disease 2019 (COVID-19) during pregnancy, vaginal delivery, and in the neonatal period. One study showed that the SARS-CoV-2 antibody titer remained stable from infection in the first trimester until delivery.[8] 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 immunoglobulin G (IgG), others found both immunoglobulin M (IgM) and IgG.[3,9,10] As IgG is the only antibody class that significantly crosses the placenta, findings of IgM in cord blood indicate in utero infection, whereas IgM in neonates could be caused by either in utero infection or postnatal infection. Additionally, the time span between maternal confirmed SARS-CoV-2 and the presence of antibodies in umbilical cord has been explored. Findings were ambiguous, as in one case IgG was found in cord blood already 1 day after expected maternal infection, which is an unusually fast IgG response.[3] Overall, previous studies may indicate that the risk of mother-to-child transmission is increased if the woman delivers less than 1 week from onset of infection.

Key message

SARS-CoV-2 might be detectable in the vagina up to 8 days after a positive pharyngeal swab, while antibodies possibly do not yet protect the neonate. Data are needed regarding seroconversion for the mother and appearance of antibodies in cord blood.

The objective of this study was to investigate if SARS-CoV-2 is detectable in vaginal swabs of pregnant women with confirmed SARS-CoV-2 infection and to study the presence and timing of SARS-CoV-2 antibodies in maternal and umbilical cord blood.

2 | MATERIAL AND METHODS

We conducted a prospective cohort study entitled the "CareMum COVID-19 study" from August 20, 2020 to March 1, 2021 at the Department of Obstetrics and Gynecology, Copenhagen University Hospital—North Zealand. The hospital has approximately 4000 annual deliveries.

We included pregnant women with confirmed SARS-CoV-2 infection based on a pharyngeal swab positive for SARS-CoV-2. The women had been tested at a public or private test center or at a routine test when entering the hospital for admission. During the

study period, the hospital had a protocol for universal screening of all patients admitted to the maternity ward or the antepartum clinic regardless of symptoms. In case of an elective cesarean section the swab was performed the day before delivery. 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 antepartum at the antenatal clinic or intrapartum when women were admitted for delivery. Vaginal swabs and maternal blood samples were collected from all participants at the time of inclusion. If included antepartum, the samples were repeated intrapartum, when an umbilical cord blood sample was also taken (Figure 1). Intrapartum, two vaginal swabs were taken—the first during the initial vaginal examination and the second during the active phase of delivery or immediately after. Vaginal swabs were stored at −20°C until analysis (0–30 days, median 5 days). The maternal blood sample was taken just before or immediately after delivery, and the umbilical cord blood sample was collected immediately after birth. Blood samples were stored 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 were performed on clinical indications (Supporting Information Table S1). Placental swabs were performed from both the maternal and the fetal side, and neonatal swabs from the axillary fold, nasopharynx, and oropharynx. These samples were not part of the study protocol and therefore not systematically sampled, but results are described when present. Pharyngeal swabs as well as vaginal and placental swabs were analyzed by reverse transcription polymerase chain reaction (RT-PCR) as part of the routine diagnostics (see Supporting Information Appendix S1, for further information).

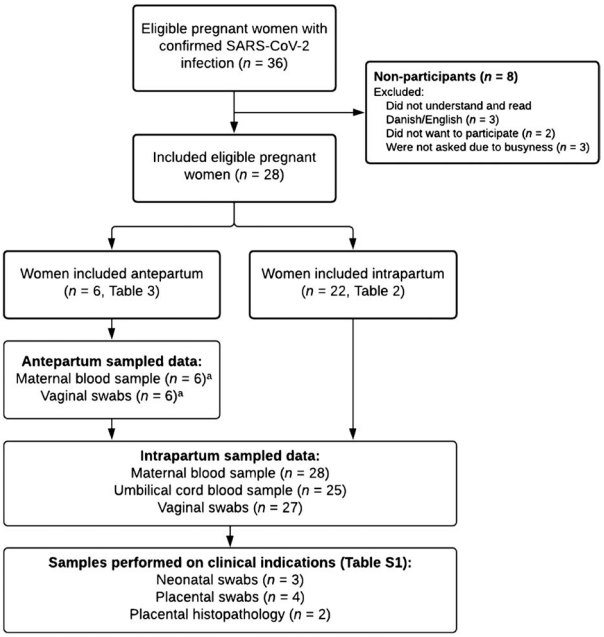

FIGURE 1 Flowchart of participants and sampled data. Data sampled antepartum included a maternal blood sample and two vaginal swabs. Data sampled intrapartum included a maternal blood sample as well as an umbilical cord blood sample and two vaginal swabs. In some cases, other samples were performed on clinical indications. [a]One woman (participant 28) had three consecutive samples (both vaginal swabs and blood samples) performed on day 1, 16, and 37 after confirmed SARS-CoV-2 infection resulting in a total of 8 vaginal swabs and 8 maternal blood samples

Total SARS-CoV-2 antibodies (including IgG and IgM) in maternal and cord blood samples were analyzed using a qualitative (reactive/non-reactive) biochemical assay, the VITROS Immunodiagnostic Product Anti-SARS-CoV-2 Total (CoV2T), developed by Ortho-Clinical Diagnostics. A test result (S/C = signal for test sample/signal at cut-off value) of 1.00 or more is considered reactive (ie, positive for antibodies) and a result below 1.00 is considered non-reactive (ie negative for antibodies). The assay has been shown to have a specificity of more than 99% and a sensitivity of 95.3%.[11]

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 with confirmed SARS-CoV-2 infection during pregnancy in Denmark, as described elsewhere.[12] Case completeness of the database was secured by a retrospective registry linkage to national databases containing information on positive SARS-CoV-2 tests. Furthermore, participants were asked to complete a questionnaire about COVID-19 symptoms at the time of inclusion.

2.1 | Statistical analyses

Data were analyzed using SPSS version 27 (SPSS Inc.). Categorical variables are presented as number with percentage and continuous variables as mean with standard deviation or median with interquartile range as appropriate.

2.2 | Ethical approval

The Regional Committee on Health Research Ethics in the capital region of Denmark approved the study on May 3, 2020 with an additional protocol approved November 17, 2020 (H-20028002). Written informed consent was collected from all participants. Demographic and clinical data were extracted from the prospective national observational study "COVID-19 in pregnancy", approved by the Danish Patient Safety Authority on April 24, 2020 (reg. no. 31-1521-252) and the regional Data Protection Agency in Region Zealand on March 23, 2020 (reg. no. REG022-2020).

3 | RESULTS

We included 28 of 36 (77.8%) women with confirmed SARS-CoV-2 infection during pregnancy in the study period. Only four women tested positive by routine screening when admitted to the maternity ward for labor, of whom one was asymptomatic. All other women were tested positive elsewhere. Non-participants (n = 8,

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
Parity, nulliparous	11 (39.3)	3 (37.5)
Ethnicity, Caucasian	15 (53.6)	4 (50.0)
Pre-existing medical disorders[a]	7 (25.0)	2 (25.0)
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)	
Dyspnea	7 (25.0)	
Diarrhea	4 (14.3)	
Asymptomatic	4 (14.3)	
Maternal admission due to COVID-19		
Medical ward	3 (10.7)	0
ICU	0	0
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		
Vaginal delivery	19 (67.9)	4 (50.0)
Vaginal, vacuum extraction	1 (3.6)	0
Elective cesarean section	4 (14.3)	1 (12.5)
Acute cesarean section	4 (14.3)	3 (37.5)
Perinatal outcomes		
Gestational age (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)
Birthweight (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

Data are presented as mean (± standard deviation), median (interquartile range) or proportions (%). For some of the variables, proportions are changing because of missing data.

BMI, body mass index; ICU, Intensive Care Unit; GA, gestational age; NICU, Neonatal Intensive Care Unit.

[a]Essential hypertension ($n = 1$), Factor V Leiden mutation ($n = 1$), β-thalassemia ($n = 1$), hypothyroidism ($n = 3$), polycystic ovary syndrome ($n = 1$), depression ($n = 2$).

Table 1) were women with confirmed SARS-CoV-2 infection who gave birth within the study period, but who were not included in this study for various reasons, eg did not understand Danish/English, did not want to participate or were not asked because of busyness (Figure 1). Twenty-two participants (78.6%) were included intrapartum (Table 2), and six participants were included and examined antepartum with samples repeated intrapartum (Table 3). Participants had confirmed SARS-CoV-2 infection between 25^{+0} and 41^{+4} weeks of gestation and 24 women (85.7%) were symptomatic at the time of testing. The onset of symptoms was reported from 16 days before to 3 days after the positive swab. 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 private test centers.

Vaginal swabs were obtained from all participants, but one woman had a vaginal swab only antepartum and not intrapartum. SARS-CoV-2 was detected in the vaginal swabs of 2 of 28 women—at 1 and 8 days, respectively, after confirmed SARS-CoV-2 infection (7.1%). One vaginal swab was inconclusive. No positive vaginal swabs were found during vaginal delivery, independent of the timing with regards to onset of symptoms or confirmed SARS-CoV-2 infection (Tables 2 and 3).

Maternal antibodies were analyzed in all 28 women (Tables 2 and 3). Within 8 days of confirmed SARS-CoV-2 infection, SARS-CoV-2 antibodies were detected in only one (participant 6, Table 2) of 13 women (7.7%), whereas antibodies were observed in 19 of 21 (90.5%) women from 16 days or more after confirmed infection (Tables 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 analyzed in 25 umbilical cord blood samples. Antibodies were not detected in umbilical cord blood from pregnancies where the woman delivered within 16 days of confirmed SARS-CoV-2 infection ($n = 7$). However, antibodies were detected in 16 of 17 (94.1%) cord blood samples of pregnancies where the woman was seropositive and delivered from day 26 after infection (Tables 2 and 3). In the time span of 9–25 days after confirmed infection, the cohort included only one woman; a woman who delivered 16 days

TABLE 2 Pregnant women with confirmed SARS-CoV-2 infection either antepartum or intrapartum—data only sampled intrapartum

	Data sampled intrapartum							
	Days from confirmed SARS-CoV-2 infection to data sampled intrapartum	Confirmed SARS-CoV-2 infection (gestational age, weeks)	Onset of symptoms (gestational age, weeks)	Delivery (gestational age, weeks)	Mode of delivery (VD/CS)	Vaginal swabs for SARS-CoV-2[a]	Total antibodies (Test result (S/C))	
Pt							Maternal	Umbilical cord
1	0	38^{+1}	No symptoms	38^{+1}	VD	–/–[b]	<1.00	N/A
2	1	37^{+5}	36^{+2}	37^{+6}	VD	–/–	<1.00	<1.00
3	1	41^{+0}	41^{+1}	41^{+1}	E-CS	–/–	<1.00	<1.00
4	1	41^{+4}	41^{+2}	41^{+5}	VD	–/–	<1.00	<1.00
5	5	29^{+1}	29^{+4}	29^{+6}	E-CS	Inconclusive	<1.00	<1.00
6	8	31^{+2}	31^{+0}	32^{+3}	E-CS	+[c]	9	<1.00
7	8	39^{+3}	39^{+3}	40^{+4}	VD	–/–	<1.00	<1.00
8	16	38^{+6}	N/A	41^{+1}	VD	–/–	3	<1.00
9	30	35^{+5}	35^{+5}	41^{+0}	VD	–/–	24	16
10	30	36^{+5}	35^{+5}	41^{+0}	VD	–/–	2	<1.00
11	35	35^{+3}	N/A	40^{+3}	VD	–/–	8	7
12	38	31^{+4}	32^{+2}	37^{+0}	VD	–/–	95	31
13	51	34^{+5}	34^{+5}	41^{+3}	VD	–/–	45	79
14	61	31^{+4}	31^{+3}	40^{+2}	VD	–/–	4	2
15	63	30^{+0}	30^{+0}	39^{+0}	VD	–/–	20	20
16	64	30^{+5}	30^{+5}	39^{+6}	VD	–/–	14	20
17	66	30^{+4}	N/A	40^{+0}	VD	–/–	34	31
18	69	30^{+6}	30^{+5}	40^{+5}	E-CS	–/–	11	N/A
19	79	28^{+5}	28^{+6}	40^{+0}	E-CS	–/–	73	115
20	86	26^{+0}	26^{+0}	38^{+2}	VD	–/–	13	13
21	91	26^{+6}	26^{+4}	39^{+6}	E-CS	–/–	42	134
22	102	25^{+0}	No symptoms	39^{+4}	VD	–/–	124	522

Abbreviations: E-CS, emergency cesarean section; N/A, not available; Pt, Participant; SARS-CoV-2, severe acute respiratory coronavirus-2; VD, vaginal delivery.

Results of swabs analyzed 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 cut-off value) ≥1.00 is considered reactive (ie positive for antibodies) and result <1.00 is considered non-reactive (ie negative for antibodies).

[a]In an emergency cesarean section, two vaginal swabs were performed: at an initial vaginal examination and immediately before/after the emergency cesarean section, respectively. In a 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.

[b]Vaginal swabs performed 1 day postpartum.

[c]Only one vaginal swab was performed due to E-CS.

TABLE 3 Pregnant women with confirmed SARS-CoV-2 infection antepartum—data sampled antepartum and intrapartum

	Data sampled antepartum					Data sampled intrapartum					
										Total antibodies (Test result (S/C))	
Pt	Days from confirmed SARS-CoV−2 infection to data sampled antepartum	Confirmed SARS-CoV−2 infection (gestational age, weeks)	Onset of symptoms (gestational age, weeks)	Vaginal swabs for SARS-CoV-2[c]	Maternal total antibodies (test result (S/C))	Days from confirmed SARS-CoV-2 infection to data sampled intrapartum	Delivery (gestational age, weeks)	Mode of delivery (VD/CS)	Vaginal swab for SARS-CoV-2[a]	Maternal	Umbilical cord
23	1	37^{+1}	No symptoms	−/−	<1.00	26	40^{+6}	VD	−/−	7	3
24	5	35^{+6}	35^{+6}	−/−	<1.00	34	40^{+5}	VD	−/−	6	10
25	1	31^{+0}	28^{+4}	−/−	<1.00	57	39^{+1}	VD	−/−	<1.00	<1.00
26	1	28^{+3}	No symptoms	−/−	<1.00	74	39^{+0}	VD	N/A	<1.00	N/A
27	8	27^{+2}	27^{+4}	−/−	<1.00	75	38^{+0}	P-CS	−/−	250	290
28	1	26^{+5}	26^{+4}	+/+	<1.00	84	38^{+5}	P-CS	−/−	484	463
	16			−/−	42						
	37			−/−	158						

Abbreviations: N/A, not available; P-CS, planned cesarean section; Pt, participant; SARS-CoV-2, severe acute respiratory coronavirus-2; VD, vaginal delivery.

Results of swabs analyzed 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 cut-off value) ≥1.00 is considered reactive (ie positive for antibodies) and result <1.00 is considered non-reactive (ie negative for antibodies).

[a]Two vaginal swabs performed at the same time.

[b]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.

after confirmed SARS-CoV-2 infection. The test results (S/C) of antibodies in maternal and cord blood at delivery were highly correlated (Supporting Information Figure S1, $R^2 = 0.64$, $p < 0.001$). There was a positive correlation between the test results (S/C) for antibodies in cord blood and the number of days from confirmed SARS-CoV-2 infection to delivery (Supporting Information Figure S2, $R^2 = 0.43$, $p = 0.002$), whereas the correlation between the test result (S/C) for antibodies in maternal blood and the number of days between confirmed SARS-CoV-2 infection and delivery was not statistically significant (Supporting Information Figure S2, $R^2 = 0.20$, $p = 0.054$). Cord blood samples from three children of 19 seropositive mothers were without detectable antibodies (15.8%), but the remaining 16 cord blood samples had detectable antibodies.

3.1 | Clinical findings and complications in pregnancies with SARS-CoV-2 infection

Participant 5 had confirmed SARS-CoV-2 infection at 29^{+1} weeks of gestation. She presented at the antenatal ward at 29^{+6} weeks of gestation with reduced fetal movements and vaginal bleeding. The neonate was delivered the same day by emergency cesarean section in response to a pathological cardiotocography (CTG). APGAR score was 8 after 5 minutes and umbilical cord blood showed respiratory compensated metabolic acidosis. Microscopic examination of the placenta showed severe acute and chronic intervillositis with necrosis of the trophoblast (Supporting Information Figure S3). The results of the vaginal swabs were inconclusive. Swabs from the fetal side of the placenta were positive for SARS-CoV-2. Maternal as well as cord blood tested negative for SARS-CoV-2 antibodies.

Participant 6 had confirmed SARS-CoV-2 infection at 31^{+2} weeks of gestation. She presented at the antenatal ward at 32^{+0} weeks of gestation with a reduction in fetal movements but was discharged after a normal CTG. At 32^{+3} weeks of gestation she presented with contractions. The neonate was delivered shortly after by emergency cesarean section because of a preterminal CTG with APGAR 0 after 5 minutes and umbilical cord blood with severe metabolic acidosis. One hour after delivery, the neonate was declared dead after a prolonged neonatal resuscitation attempt. Autopsy showed no malformations, but signs of asphyxia from petechiae and meconium aspiration. There was some histological 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 (Supporting Information Figure S4). SARS-CoV-2 was detected in the vaginal swab at delivery and in swabs from both the fetal and maternal sides of the placenta. Neonatal swabs were SARS-CoV-2 negative. The mother tested positive for SARS-CoV-2 antibodies but umbilical cord blood tested negative. Blood and urine culture at the time of delivery were negative.

Altogether, SARS-CoV-2 positive swabs from the placenta and placental examinations with findings of severe fibrin deposit indicate the involvement of SARS-CoV-2 infection as part of the pathogenesis. By reference to a systematic classification system, the above-mentioned cases could be classified as "probable congenital infections" because the virus was detected by PCR in the placental swabs from the fetal side of the placenta in neonates delivered by cesarean section before rupture of membranes.[13]

Besides the two severe neonatal cases described, few maternal complications were found (Table 1). Swabs from the neonates were taken in two cases (participant 3 and 5, Supporting Information Table S1), neither was positive for SARS-CoV-2. No children had confirmed SARS-CoV-2 infection after birth.

4 | DISCUSSION

In this prospective cohort study, we found SARS-CoV-2 in 2 of 28 vaginal swabs up to 8 days after confirmed SARS-CoV-2 infection. SARS-CoV-2 total antibodies were detected in maternal blood samples from day 8 and in the majority from 16 days after confirmed infection. In cord blood, antibodies were detected from 26 days. Reduced fetal movements combined with abnormal CTG and placental findings associated with fetal asphyxia were observed in two cases.

Our study indicates that the neonate is not protected against infection in the acute phase of maternal SARS-CoV-2 infection, as between 0 and 25 days (after a positive maternal pharyngeal swab), there were no SARS-CoV-2 antibodies in cord blood even though the mother had produced antibodies. However, from 26 days after confirmed SARS-CoV-2 infection, 94.1% of neonates had antibodies in cord blood. Other studies have assessed the time span between maternal SARS-CoV-2 diagnosis and the presence of antibodies in cord blood. In one study, where antibodies were identified in 11 of 31 cord blood samples, one case had IgG 1 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 being diagnosed. Another study found that 11 infants of 83 seropositive mothers (13%) did not have antibodies in cord blood after a median time of 6 days (interquartile range 0–12 days) from diagnosis to delivery.[14] This time span for detection of antibodies in cord blood is shorter than the 26 days we report. However, our study only includes one woman giving birth between days 9 and 25 after confirmed infection. The woman (participant 8, Table 2) had positive antibodies, but no antibodies were detected in cord blood 16 days after confirmed maternal infection. In accordance with our study, there was a positive correlation between the levels of maternal and cord-blood IgG.[14] In our study, two cases did not have antibodies in either maternal or umbilical cord blood (participants 25 and 26). The pharyngeal swab of participant 25 had a low cycle threshold value, but a negative swab 4 days later, while participant 26 had no symptoms, a pharyngeal swab with a high cycle threshold value, and a negative swab the day after testing positive. Possible explanations include either a false-positive swab, a false-negative antibody test, 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 time span from SARS-CoV-2 infection to delivery (8 and 16 days, respectively).

In contrast to our study, most previous studies report negative vaginal swabs for SARS-CoV-2.[1,15–18] However, there are reports of SARS-CoV-2 detected in vaginal swabs ($n = 5$) in three pregnant women, indicating that transmission during vaginal delivery hypothetically might be possible, although the risk seems low, and a positive SARS-CoV-2 PCR does not prove infectious potential.[3–6] There are several potential sources for viral presence in the vagina. The coronavirus binds to target cells through angiotensin-converting enzyme 2 receptors, which are upregulated in vaginal epithelium during pregnancy, making it possible for the virus to bind.[19] 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.[20] Previous studies have also reported positive placental swabs, indicating a potential viral spread either as an ascending infection or through the bloodstream ($n = 5$).[3,4,6,21] Other studies have demonstrated SARS-CoV-2 in neonatal swabs of children born to women with symptoms of COVID-19,[15,16,22,23] which we did not find. The clinical implications of positive vaginal swabs for SARS-CoV-2 in pregnancy need further investigation.

The adverse fetal outcomes of participants 5 and 6 in our study are similar to a case of suspected transplacental transmission of SARS-CoV-2 from Sweden, where a woman with a 3-day history of COVID-19 presented with reduced fetal movements at 34^{+4} weeks of gestation.[7] After an emergency cesarean section for a pathological CTG, the mother and child were separated. Placental pathology was similar to the findings in our study as well as other studies examining placentas from women with confirmed SARS-CoV-2 infection.[4,7,24–27] Authors concluded that the neonate had suffered from transient asphyxia attributed to intrauterine hypoxia secondary to placental dysfunction. The case from Sweden and others, as well as participants 5 and 6 in our study, highlight that reduced fetal movements should always be handled with concern and that women should seek immediate care at any such occurrence.[4,7]

The study has several strengths, including the prospective design. Furthermore, 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 confirmed SARS-CoV-2 infection 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 centers reporting the results to national databases, which may reduce the number of undetected cases. Finally, we used a serological assay with a high diagnostic accuracy with a sensitivity of 95.3% and a specificity of 100%.[11]

The study also has limitations. We included 77.8% of eligible women and had a small sample size, although larger than some previous studies examining vaginal swabs.[17,18] As a result of the small sample size, the study includes a limited number of blood samples, covering only fragments of the period after confirmation of the SARS-CoV-2 infection. We can therefore not conclude on the exact timing of seroconversion, but only estimate a time span for seroconversion as well as the appearance of antibodies in cord blood.

Most vaginal swabs were kept at −20°C until analysis, possibly causing a deterioration of SARS-CoV-2 RNA 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 −20°C for up to 84 days.[28] Lack of infection status from the participants' partners is also a limitation, as it is not fully evaluated whether SARS-CoV-2 can be transmitted through semen and cause detection of SARS-CoV-2 in the vagina.[20] We measured total SARS-CoV-2 antibodies and therefore were not able to discriminate between IgG and IgM. However, for an individual, specific SARS-CoV-2 IgG/IgM assays may be difficult to interpret because of a significant variation in the IgM and IgG antibody response to SARS-CoV-2. Hence, measuring total antibodies improves sensitivity.[29,30] The assay used in this study targets the spike protein, and the presence of spike protein antibodies is indicative of either previous infection or vaccination.[31] In this study none of the women had been vaccinated; consequently, a positive antibody test indicates previous infection. Although nucleoprotein antibodies are known to be more abundant compared with spike protein antibodies and so assays targeting nucleoprotein may be more sensitive for earlier detection of infection, anti-nucleoprotein antibody responses have been found to wane in the post infection phase in contrast to anti-spike protein antibody responses.[30] Further, concerns have been raised about the specificity of the use of nucleoprotein for serological assays due to homology with nucleoproteins of other coronaviruses.[32] This is particularly important in a low-incidence population such as in the present study. Three participants were not tested by pharyngeal RT-PCR (one by an antigen test and two at private test centers), so exact details about analysis methods are lacking. However, these three participants were positive for SARS-CoV-2 antibodies, confirming previous infection. Finally, neonatal and placental examinations were not systematically collected and were only performed on clinical indications, limiting the generalizability of these results.

In large cohort studies, the overall risk of severe neonatal outcomes as well as neonatal infection related to maternal SARS-CoV-2 infection seems low, however, a recent paper reports increased rates of fetal death, preterm birth, preeclampsia, emergency cesarean delivery, and other adverse maternal and neonatal outcomes.[33] High-quality evidence syntheses of comparative studies as well as studies describing detailed clinical observations from cases with adverse outcomes are needed to guide future clinical decisions in pregnancies complicated by COVID-19.[34]

5 | CONCLUSION

In pregnant women with confirmed SARS-CoV-2, virus was detected in only 2 of 28 vaginal swabs within 8 days after confirmed infection.

Our data suggest that maternal seroconversion occurs between days 8 and 16 after confirmed infection, whereas antibodies in cord blood of seropositive mothers were present in the majority from 26 days after confirmed infection. Data are still needed regarding timing of seroconversion for the mother and appearance of antibodies in cord blood. Also, the clinical implications of SARS-CoV-2 in the vagina need further evaluation.

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AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

None.

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REFERENCES

1. Chi J, Gong W, Gao Q. Clinical characteristics and outcomes of pregnant women with COVID-19 and the risk of vertical transmission: a systematic review. *Arch Gynecol Obstet*. 2021;303:337-345.
2. Kotlyar AM, Grechukhina O, Chen A, et al. Vertical transmission of coronavirus disease 2019: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2021;224:35-53.e3.
3. Fenizia C, Biasin M, Cetin I, et al. Analysis of SARS-CoV-2 vertical transmission during pregnancy. *Nat Commun*. 2020;11:5128.
4. Schoenmakers S, Snijder P, Verdijk RM, et al. Severe Acute Respiratory Syndrome Coronavirus 2 placental infection and inflammation leading to fetal distress and neonatal multi-organ failure in an asymptomatic woman. *J Pediatric Infect Dis Soc*. 2021;10:556-561.
5. Schwartz A, Yogev Y, Zilberman A, et al. Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in vaginal swabs of women with acute SARS-CoV-2 infection: a prospective study. *BJOG*. 2021;128:97-100.
6. Vivanti AJ, Vauloup-Fellous C, Prevot S, et al. Transplacental transmission of SARS-CoV-2 infection. *Nat Commun*. 2020;11:3572.
7. Zaigham M, Holmberg A, Karlberg ML, et al. Intrauterine vertical SARS-CoV-2 infection: a case confirming transplacental transmission followed by divergence of the viral genome. *BJOG*. 2021;128:1388-1394.
8. Cosma S, Carosso AR, Corcione S, et al. Longitudinal analysis of antibody response following SARS-CoV-2 infection in pregnancy: from the first trimester to delivery. *J Reprod Immunol*. 2021;144:103285.
9. Dong L, Tian J, He S, et al. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn. *JAMA*. 2020;323:1846-1848.
10. Zeng H, Xu C, Fan J, et al. Antibodies in infants born to mothers with COVID-19 pneumonia. *JAMA*. 2020;323:1848-1849.
11. Harritshoj LH, Gybel-Brask M, Afzal S, et al. Comparison of sixteen serological SARS-CoV-2 immunoassays in sixteen clinical laboratories. *J Clin Microbiol*. 2021;59:e02596-e2620.
12. Aabakke AJM, Krebs L, Petersen TG, et al. SARS-CoV-2 infection in pregnancy in Denmark-characteristics and outcomes after confirmed infection in pregnancy: a nationwide, prospective, population-based cohort study. *Acta Obstet Gynecol Scand*. 2021 Aug 31. doi: 10.1111/aogs.14252. Epub ahead of print.
13. Shah PS, Diambomba Y, Acharya G, Morris SK, Bitnun A. Classification system and case definition for SARS-CoV-2 infection in pregnant women, fetuses, and neonates. *Acta Obstet Gynecol Scand*. 2020;99:565-568.
14. Flannery DD, Gouma S, Dhudasia MB, et al. Assessment of maternal and neonatal cord blood SARS-CoV-2 antibodies and placental transfer ratios. *JAMA Pediatr*. 2021;175:594-600.
15. Debrabandere ML, Farabaugh DC, Giordano C. A review on mode of delivery during COVID-19 between December 2019 and April 2020. *Am J Perinatol*. 2021;38:332-341.
16. Neef V, Buxmann H, Rabenau HF, Zacharowski K, Raimann FJ. Characterization of neonates born to mothers with SARS-CoV-2 infection: review and meta-analysis. *Pediatr Neonatol*. 2021;62:11-20.
17. Aslan MM, Uslu Yuvaci H, Kose O, et al. SARS-CoV-2 is not present in the vaginal fluid of pregnant women with COVID-19. *J Matern Fetal Neonatal Med*. 2020;6:1-3. doi: 10.1080/14767058.2020.1793318. Epub ahead of print.
18. Qiu L, Liu X, Xiao M, et al. SARS-CoV-2 is not detectable in the vaginal fluid of women with severe COVID-19 infection. *Clin Infect Dis*. 2020;71:813-817.
19. Narang K, Enninga EAL, Gunaratne MDSK, et al. SARS-CoV-2 infection and COVID-19 during pregnancy: a multidisciplinary review. *Mayo Clin Proc*. 2020;95:1750-1765.
20. Li D, Jin M, Bao P, Zhao W, Zhang S. Clinical characteristics and results of semen tests among men with Coronavirus Disease 2019. *JAMA Netw Open*. 2020;3:e208292.
21. Patanè L, Morotti D, Giunta MR, 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 MFM*. 2020;2:100145.
22. Di Toro F, Gjoka M, Di Lorenzo G, et al. Impact of COVID-19 on maternal and neonatal outcomes: a systematic review and meta-analysis. *Clin Microbiol Infect*. 2021;27:36-46.
23. Correia CR, Marçal M, Vieira F, et al. Congenital SARS-CoV-2 infection in a neonate with Severe Acute Respiratory Syndrome. *Pediatr Infect Dis J*. 2020;39:e439-e443.
24. Schwartz DA, Morotti D. Placental pathology of COVID-19 with and without Fetal and neonatal infection: trophoblast necrosis and chronic histiocytic intervillositis as risk factors for transplacental transmission of SARS-CoV-2. *Viruses*. 2020;12:1308.
25. Schwartz DA, Baldewijns M, Benachi A, et al. Chronic histiocytic intervillositis with trophoblast necrosis are risk factors associated with placental infection from Coronavirus Disease 2019 (COVID-19) and intrauterine maternal-fetal Severe Acute Respiratory Syndrome

Coronavirus 2 (SARS-CoV-2) transmission in liveborn and stillborn infants. *Arch Pathol Lab Med*. 2021;145:517-528.
26. Debelenko L, Katsyv I, Chong AM, Peruyero L, Szabolcs M, Uhlemann AC. Trophoblast damage with acute and chronic intervillositis: disruption of the placental barrier by severe acute respiratory syndrome coronavirus 2. *Hum Pathol*. 2021;109:69-79.
27. Linehan L, O'Donoghue K, Dineen S, White J, Higgins JR, Fitzgerald B. SARS-CoV-2 placentitis: an uncommon complication of maternal COVID-19. *Placenta*. 2021;104:261-266.
28. Hokajärvi A-M, Rytkönen A, Tiwari A, et al. The detection and stability of the SARS-CoV-2 RNA biomarkers in wastewater influent in Helsinki, Finland. *Sci Total Environ*. 2021;770:145274.
29. Bauer G. The variability of the serological response to SARS-corona virus-2: potential resolution of ambiguity through determination of avidity (functional affinity). *J Med Virol*. 2021;93:311-322.
30. Fenwick C, Croxatto A, Coste AT, et al. Changes in SARS-CoV-2 spike versus nucleoprotein antibody responses impact the estimates of infections in population-based seroprevalence studies. *J Virol*. 2021;95:e01828-e1920.
31. Houlihan CF, Beale R. The complexities of SARS-CoV-2 serology. *Lancet Infect Dis*. 2020;20:1350-1351.
32. Egia-Mendikute L, Bosch A, Prieto-Fernández E, et al. Sensitive detection of SARS-CoV-2 seroconversion by flow cytometry reveals the presence of nucleoprotein-reactive antibodies in unexposed individuals. *Commun Biol*. 2021;4:486.
33. Gurol-Urganci I, Jardine JE, Carroll F, et al. Maternal and perinatal outcomes of pregnant women with SARS-CoV-2 infection at the time of birth in England: national cohort study. *Am J Obstet Gynecol*. 2021 May 20:S0002-9378(21)00565-2. doi: 10.1016/j.ajog.2021.05.016. Epub ahead of print.
34. Vergara-Merino L, Meza N, Couve-Pérez C, et al. Maternal and perinatal outcomes related to COVID-19 and pregnancy: an overview of systematic reviews. *Acta Obstet Gynecol Scand*. 2021;100:1200-1218.

SUPPORTING INFORMATION

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