REVIEW

Mechanisms and evidence of vertical transmission of infections in pregnancy including SARS-CoV-2s

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Funding information
National Medical Research Council, Singapore., Grant/Award Number: Clinician-Scientist Award NMRC/CSA/059/2014.

Abstract

There remain unanswered questions concerning mother-to-child-transmission of SARS-CoV-2. Despite reports of neonatal COVID-19, SARS-CoV-2 has not been consistently isolated in perinatal samples, thus definitive proof of transplacental infection is still lacking. To address these questions, we assessed investigative tools used to confirm maternal-fetal infection and known protective mechanisms of the placental barrier that prevent transplacental pathogen migration. Forty studies of COVID-19 pregnancies reviewed suggest a lack of consensus on diagnostic strategy for congenital infection. Although real-time polymerase chain reaction of neonatal swabs was universally performed, a wide range of clinical samples was screened including vaginal secretions (22.5%), amniotic fluid (35%), breast milk (22.5%) and umbilical cord blood. Neonatal COVID-19 was reported in eight studies, two of which were based on the detection of SARS-CoV-2 IgM in neonatal blood. Histological examination demonstrated sparse viral particles, vascular malperfusion and inflammation in the placenta from pregnant women with COVID-19. The paucity of placental co-expression of ACE-2 and TMPRSS2, two receptors involved in cytoplasmic entry of SARS-CoV-2, may explain its relative insensitivity to transplacental infection. Viral interactions may utilise membrane receptors other than ACE-2 thus, tissue susceptibility may be broader than currently known. Further spatial-temporal studies are needed to determine the true potential for transplacental migration.

1 | INTRODUCTION

The current COVID-19 pandemic raises many concerns about its effect on pregnancy.[1] Although SARS-CoV-2 infection causes serious complications in individuals with immune deficiencies and certain co-morbidities, pregnancy does not constitute a risk factor for severe manifestations of SARS-CoV-2.[1,2] Additionally, the fetus may be at risk of intrauterine or perinatal infection; although the question of transplacental infection (vertical transmission) of SARS-CoV-2 has not been conclusively answered, reports of neonatal infection with

COVID-19 shortly after delivery suggest either transplacental migration, or horizontal transmission via direct surface contact at delivery or during breastfeeding as the mode of viral migration.[3] To establish the case for transplacental infection, there are two important considerations: a robust diagnostic strategy to confidently confirm maternal, fetal and neonatal infections and an understanding of pathogen-specific functional biology at the maternal-fetal placental interface that determines viral toxicity, host responses towards infection and transmission mechanics. In this review, we summarise the latest in SARS-CoV-2 diagnostics applied in pregnancy and provide a mechanistic description of virus-host interactions at the placental interface to determine the risk of intrauterine infection.

2 | IMMUNE DEFENCES AT THE MATERNAL-FETAL INTERFACE AND TRANSPLACENTAL INFECTIONS

2.1 | The placenta protects the fetus from infections

The placenta is a physical and immunological defence against fetal infection (Figure 1). Maternal natural killer (NK) cells, decidual macrophages and T cells co-inhabit the placental decidua. Immune cells in the decidua are vital in placental remodelling and implantation; deficiencies are associated with miscarriage and other adverse pregnancy outcomes.[4] Although decidual NK cells are restrained by decidual macrophages via cytokines to prevent cytotrophoblast injury,[5] this protective mechanism may predispose the placenta to infections which would normally trigger a NK cell response. Decidual macrophages perform anti-microbial functions,[6] and placental T cells regulate fetal-maternal tolerance, whereas decidual virus-specific CD8^{+} T cells protects the fetus from infection.[7] Syncytiotrophoblasts (SCTs) and cytotrophoblasts (CTs) act as additional barriers to traversing infections and demonstrate varying resistance to pathogens,[8,9] mediated by Toll-like receptors which regulate expression of anti-microbial pathways via interferon-β and secretory leukocyte protease inhibitor.[10] Anti-microbial peptides have been identified in the placenta,[11] accounting for protection against transplacental transmission of various pathogens.

What is already known about this topic?

- There are reports of possible transplacental infection of SARS-CoV-2 with positive virions isolated from amniotic fluid, neonatal nasopharyngeal swabs, placenta and positive serology of neonates, but their actual validity in proving mother-to-child-transmission is still uncertain.

What does this study add?

- Provides an overview of the biology of the maternal-fetal interface, and the mechanisms involved the vertical viral transmission including SARS-CoV-2.
- Summarises the diagnostics applied in pregnancy to assess presence of mother-to-child-transmission.

When the maternal-fetal interface barrier fails, pathogens breach the innate maternal immune system and placental trophoblastic host defence to infect the fetus by mechanisms not completely elucidated. Herpesviruses (varicella, cytomegalovirus [CMV]), rubivirus (rubella), flaviviruses (hepatitis C, dengue, zika virus [ZIKV]), hepadnavirus
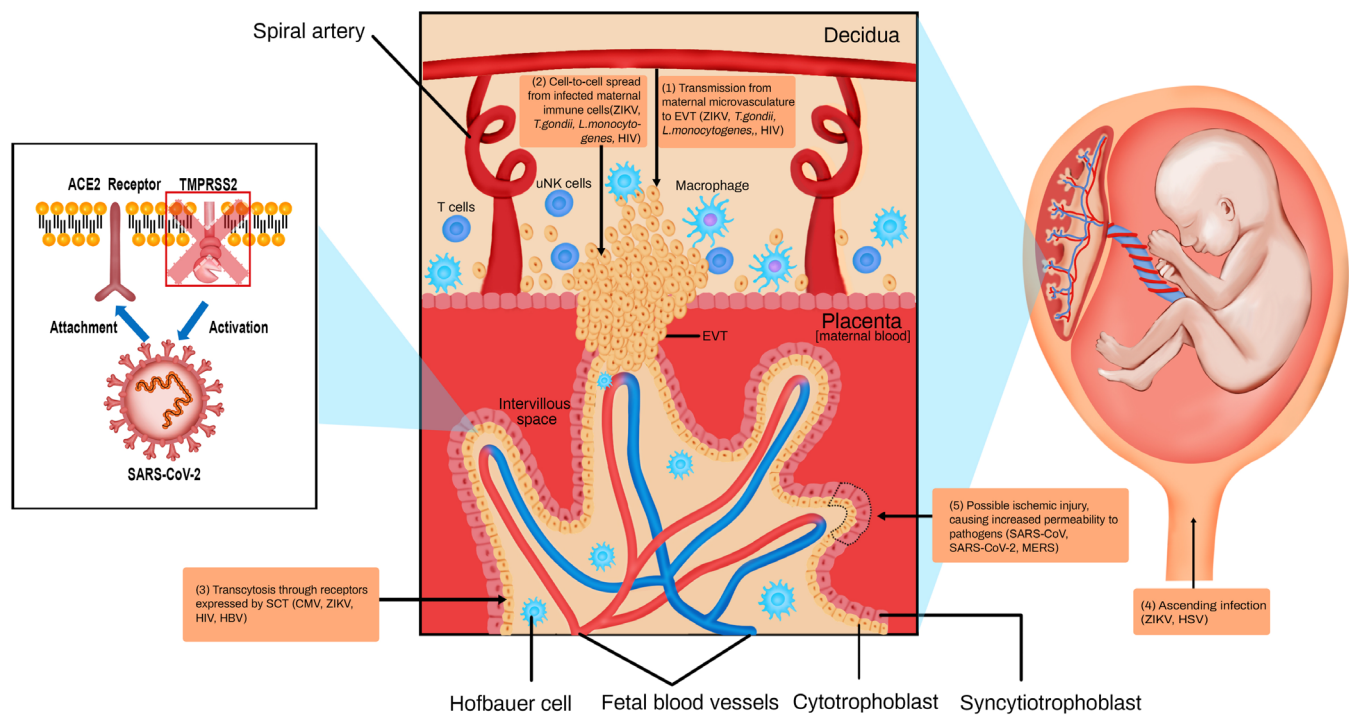
FIGURE 1 The placenta is a physical and immunological barrier. The main (middle) panel shows the cellular constituents and architecture of the maternal-fetal interface. These comprise a microscopic section of the gross placenta, which is shown, for orientation, together with the fetus inside the uterine cavity (right panel). The possible routes of pathogen migration from mother to fetus, and across the placental barrier, are described in the coloured boxes (1-5). The left panel is an expanded view of the cell membrane from a chorionic villus cell depicting the presence of ACE-2 receptor, and the absence of TMPRSS2 in this cell type (the putative location is boxed in and crossed-out). Co-expression of both ACE-2 and TMPRSS2 is required for SARS-CoV-2 virion entry into the cell cytoplasm

TABLE 1 Summary of intrauterine infectious pathogens, their route of entry and its effects on the fetus and placenta

Pathogen	Route of entry	Effects on fetus	Effects on placenta
Rubella	Placenta	Cataracts, cardiac defects, deafness, microcephaly, IUGR, CNS abnormalities, hepatosplenomegaly and bone lesions.[12]	Hypoplasia, placentitis, lobular rarefaction, dysmaturity of villous trunci and villus, villitis, villi agglutinated by fibrin and inclusion bodies in fetal and decidual cells.[13]
Cytomegolovirus	Placenta via cytotrophoblast or invasive cytotrophoblast.[14]	Mental retardation, vision loss, sensorineural deafness, prematurity and IUGR.	Underdevelopment of the placenta, CMV impairs cytotrophoblast differentiation/invasion, impairs formation of floating and anchoring villi leading to reduced surface area of villous tree. Clusters of cytomegalic cells in villi, massive villous destruction and villitis and fibrotic areas with pigment macrophages and thrombus.[14]
Parvovirus B19	Placenta	Fetal loss, hydrops fetalis (non-immune),[15] congenital anomalies (CNS, craniofacial, eye) and fetal anaemia.	Chronic villitis, chorioamnionitis, viral inclusions. Infarction and necrosis, villous oedema, villus immaturity and increased erythropoiesis.[16]
Varicella-Zoster	Placenta Ascending infection from the cervix.[17]	Abortion, stillbirth, congenital anomalies, cataract, skin lesions, CNS damage, cranial calcifications and skeletal anomalies.	Diffuse basal chronic villitis, widespread infiltration of lymphocytes, histiocytes and multinucleated giant cell.[18]
Enteroviruses	Placenta: Coxsackie B-3.[19]	Stillbirth, possible congenital anomalies, hand-foot-and-mouth disease, hepatitis, meningoencephalitis, myocarditis, pneumonia, coagulopathy and rashes.[19]	Coxsackie B-3 placenta – inflammation of villi, chronic monocytic villitis, increase in Hofbauer cells and presence of myeloid cell populations.[19]
Human Immunodeficiency Virus (HIV)	Placenta cells expressing CD4, Fc receptors on syncytiotrophoblast, Hofbauer cells, placental tears and chorioamnionitis.[20] Contact with maternal secretions	Abnormalities in the thymus and spontaneous fetal loss.[21]	Placenta of fetal demise and fetus HIV positive: acute and chronic deciduitis, endometritis, areas of infarct and haematoma, small or oedematous placenta. Placenta of fetal demise and fetus HIV negative: funisitis, acute and chronic deciduitis and chorioamnionitis.[21]
MERS	Droplet, airborne transmission	Fetal demise (27%)[22] preterm delivery	Placental abruption, which can be caused by maternal infection.[23]
SARS-CoV-1	Droplet, airborne transmission.	Spontaneous miscarriage (first trimester), preterm delivery.[24] Oligohydramnios, IUGR and small for gestational age.[25]	Placentae showed greater amounts of subchorionic, intervillous and perivillous fibrin, avascular fibrotic villi, perivillous calcification, accelerated villous maturation and areas of infarct.[25]
SARS-CoV-2	Droplet, airborne transmission.	Increased incidences of preterm births; higher rates of miscarriage, perinatal death, pre-eclampsia, caesarean section deliveries; and no increased incidences of fetal growth restriction compared to general population.[26] Intrauterine fetal distress, PROM, low birth weight, feeding intolerance and respiratory distress.	Low-grade fetal vascular malperfusion.[27] Acute chorioamnionitis and fusinitis.[28] Infiltration of macrophages and T-lymphocytes. Widespread perivillous fibrin. Maternal side shows presence of decidual vasculopathy and fetal side shows mature edematous chorionic villi. Viral particles identified within the cytosol of placental cells such as syncytiotrophoblast on electron microscopy.[29,30]
Human T-cell Leukemia Virus −1	Breast feeding (major) Intrauterine and intrapartum less likely.	Miscarriage, prematurity and low birth weight.	Increase cell apoptosis, infection of trophoblast.[31]

(Continues)

TABLE 1 (Continued)

Pathogen	Route of entry	Effects on fetus	Effects on placenta
Hepatitis C	1.Intrauterine-transplacental Crossing placental barrier Leakage of cells into fetal circulation 2.During delivery (major)	Chronic hepatitis C infection[32]	Cross placental barrier, receptor mediated entry and infection of trophoblasts, injury to the placental barrier.[33]
Hepatitis B	1.Intrauterine-transplacental Crossing placental barrier. Leakage of cells into fetal circulation 2.During delivery (major) 3.Close contact with mother post-partum	Chronic carrier state, cirrhosis and hepatocellular carcinoma[34,35]	Cross-placental barrier. Virus able to gain access and replicate in all cells of the maternal-fetal interface.[36]
Lassa fever	Intrauterine	Miscarriage, intrauterine death, stillbirth.[37]	Replication in placental cells.[37]
Japanese Encephalitis	Intrauterine	Miscarriage, stillbirth.[38]	Infection and replication in placental cells.[38]
Zika virus	Intrauterine	Microcephaly, ventriculomegaly, intracranial calcification.[39] Malformations of cortical development, abnormalities of corpus callosum and posterior fossa, eye abnormalities, arthrogryposis, anaemia and IUGR. Infants may present with neurological, motor and auditory problems and epilepsy.[40]	Infect and multiply in resident macrophages (Hofbauer cells) and endothelial cells of placenta.[41]
Toxoplasmosis	Intrauterine	Spontaneous abortion and fetal loss, CNS calcifications, CNS/cranial abnormalities, retinochoroiditis.[42]	Infection of placenta, low grade chronic villitis, mononuclear inflammatory infiltrate.[43]
Herpes-Simplex	Intrapartum (majority) Intrauterine (less)	Congenital infection (rare), abortion, stillbirth, congenital anomalies (eye, CNS, skeletal), growth restriction.[44]	Inflammation of placental cells affects placentation. Infection of extravillous trophoblast cells. Syncytiotrophoblast prevents entry of virus limiting transplacental transmission.[45]

Abbreviations: CNS, central nervous system; IUGR, intrauterine growth restriction; PROM, premature rupture of membrane.

(hepatitis B), lentivirus (human immunodeficiency virus), parvoviruses, *Toxoplasma gondii* and *Listeria monocytogenes* are capable of circumventing placental defences to cause detrimental and sometimes lethal effects on the fetus (Table 1). These effects include target-organ damage (microcephaly, intracerebral calcifications, hepatosplenomegaly, chorioretinitis, microphthalmia and deafness), fetal compromise (miscarriage, growth restriction, haemolytic anaemia and hydrops) and death.[46]

2.2 | Viral transplacental migration

Pathogens can traverse the placenta and migrate from mother to fetus in several ways (Figure 1)[8,47]:

a. Maternal endothelial microvasculature to endovascular extravillous trophoblasts (EVTs).
b. Infected maternal immune cell to placental trophoblast spread (cell-to-cell).
c. Transcytosis of virions via immune-mediated receptors.
d. Transvaginal ascending infection.

EVTs form cell columns at the ends of anchoring villi, which are in direct contact with maternal immune cells and vasculature. Pathogens such as *T. gondii* first infect maternal decidual immune cells and are then transferred to proximal EVTs, which act as vectors to allow downstream transmission to the villous core and fetal vasculature.[48,49] Viruses may possess the ability to replicate in various cell types within the maternal-fetal interface. In the case of ZIKV, evidence of viral replication was identified in proliferating villus CTs, invasive CTs and Hofbauer cells (fetal macrophages) in the villous core.[41] Additionally, the ability of ZIKV to be transmitted sexually allows it to bypass the trophoblast layer via ascending infection of the amniochorionic membrane.[50] CMV replicates in multiple cell types, including decidual maternal macrophages, dendritic cells and CTs, allowing cell-to-cell spread.[51,52] Furthermore, virions may cross the placenta by transcytosis via receptors such as neonatal Fc receptors expressed by SCT that mediate IgG transport.[53] Recently, electron microscopy observations of virions invading SCT, microvillus and cell processes of fibroblast have contributed to the building evidence of SARS-CoV-2 infecting the placenta. However, as fetal tissues were virus free, this finding still does not confirm transplacental infection.[29,54] Transplacental migration as described here is distinguished

from direct, or 'mechanical', transfer of virions, as might occur in the scenarios of antenatal procedures such as amniocentesis,[55-58] preterm birth and peripartum haemorrhage.[59] Infections by this route are likely due to maternal blood trafficking into the fetal compartment, and no molecular pathogenic aetiology is invoked.

2.3 | Gestation at exposure influences fetal risk

The risk of fetal infection depends on gestation at exposure.[48] Although differentiated SCT forms a formidable layer of placental defence, deficiency in the first and second trimesters predispose to increased transplacental infectivity of certain pathogens in early pregnancy.[60] Dynamic fluctuation of the placental immune milieu throughout pregnancy may contribute to changing fetal susceptibility.[61] SCT integrity may be breached in later gestations by haemodynamic shear stress, hypoxic injury or maternal immune-mediated injury, which further facilitates maternal-fetal microbial transmission.[62,63] Even if the virus reaches the fetus through a breach in the maternal-fetal interface, fetal innate and adaptive antiviral response towards the virus may neutralise it. For example, fetal NK cells are antiviral effector cells that demonstrate activity in the liver as early as 9 weeks' gestation.[64] This may ameliorate any fetal infection and prevent or mitigate clinical sequelae.

2.4 | Investigations to demonstrate transplacental infection

Clinical proof of transplacental viral migration requires isolation of viral nucleic acids in fetal or placental tissues within the sterile intrauterine environment and/or in the newborn,[65] and adequate exclusion of horizontal transmission, direct or mechanical transfer of virions, or contamination by genital tract fluids during vaginal delivery. Diagnostic tests thus need to include an adequate range of biological samples from both mother and neonate, such as amniotic fluid, umbilical cord blood (UCB), vaginal secretions, placenta, neonatal nasopharyngeal swabs, rectal swabs and serum. Table 2 summarises the biological samples that can be collected to diagnose transplacental infection. Real-time polymerase chain reaction (RT-PCR) is the method of choice for isolating SARS-CoV-2 nucleic acids. There are a wide range of sensitivities reported for SARS-CoV-2 detection isolated from various biological samples at 29%, 63% and 93% for faeces, nasal swabs and bronchoalveolar lavages, respectively.[66] Recent studies suggest that saliva testing may be more sensitive for maternal SARS-CoV-2 infection than nasopharyngeal swabs with easier self-collection.[67,68] Thus, to confirm transplacental infection for SARS-CoV-2, analysing different biological samples by RT-PCR addresses these varied sensitivities and improves detection. With the exception of UCB, neonatal serum and surgically collected amniotic fluid,[28,69,70] other perinatal samples are susceptible to contamination by vaginal fluid containing SARS-CoV-2 or by respiratory droplets during mother-child contact after birth. In the event of vaginal delivery, which should be the mode of

delivery barring obstetric contraindications,[71] sample contamination can be minimised by (1) collecting a comprehensive range of samples immediately at birth before further contact with the mother, and (2) performing tests on the baby as soon as possible. Neonatal serum, urine and swabs from the upper or lower airway, body surface and rectum should ideally be analysed with paired maternal nasopharynx, rectum and vaginal swabs to document presence of the virus in maternal samples and to exclude possible contamination.[66,72] However, RT-PCR sensitivity for swabs of maternal vaginal secretions and neonatal surfaces has not yet been determined. SARS-CoV-2 can shed from multiple anatomical sites throughout convalescence, and rectal shedding is reportedly more prevalent in the later stages of COVID-19.[73] Sample collection should therefore reflect this evolving clinical course.

Serological assays may be useful. Antigen-specific immunoglobulin (Ig)-M in neonatal serum is considered a potential marker of intrauterine infection in congenital rubella or cytomegalovirus (CMV).[74] As the ~900 kDa size negates transplacental migration, neonatal IgM expression suggests fetal seroconversion following viral exposure. The SARS-CoV-2 IgM immunoassay's sensitivity and specificity were initially reported by the manufacturer as 70.2% and 96.2%, respectively,[75] although later studies reported 48.1% and 100%, respectively.[76] In both reports, nasopharyngeal RT-PCR were negative. Although IgM is a potentially useful marker to confirm SARS-CoV-2 transplacental infection, it shares limitations common to all serological assays such as false positive or negative results and cross-reactivity.[77,78]

3 | WHAT DO WE KNOW ABOUT MOTHER-TO-CHILD-TRANSMISSION OF SARS-COV-2?

3.1 | Case series of SARS-CoV-2 infection in pregnancy

A search was conducted in PubMed on 19 May 2020 to identify published case reports and series involving COVID-19 in pregnancy and puerperium using combinations and variations of the following search terms: SARS-CoV-2, COVID-19, nCoV, coronavirus, pregnancy, antenatal, maternal morbidity OR mortality, neonatal morbidity OR mortality, in utero infection, transplacental infection and transplacental transmission. References from each article were also manually searched for relevant studies. Screened articles included not just those published in English but also in Mandarin given that a large body of early literature came from the People's Republic of China (PRC). We studied 40 case reports and series (Table 3) documenting diagnostics performed to determine mother-to-child-transmission (MTCT). The majority are retrospective studies and originate from PRC. Although most pregnant women were diagnosed with SARS-CoV-2 by RT-PCR, a minority were diagnosed based on clinical history and radiological evidence of respiratory disease on chest computed tomography alone. Thirty-seven studies reported cases in the third trimester,

TABLE 2 Biological samples to aid in diagnosis of transplacental infection

	Samples		
	Maternal	Fetal/neonatal	
Antenatal	...	AF (amniocentesis) for RT-PCR	
First trimester miscarriage	Vaginal swab for RT-PCR	Products of conception for RT-PCR	
Second trimester miscarriage/stillbirth	Vaginal swab for RT-PCR	For RT-PCR: -Placenta -UCB -Umbilical cord -Fetal tissues[a] (eg, the heart, liver and lung)	For histological examination: -Placenta
Live birth	Intrapartum vaginal, rectal, nasopharyngeal swab for RT-PCR	Perinatal samples for RT-PCR: -AF collected prior to rupture of membranes or at caesarean-section aspirated through intact amniotic membrane after the uterus is incised -Placenta -UCB -Umbilical cord -Neonatal airway, surface, rectal swab -Neonatal SARS-CoV-2 IgM	For histological examination: -Placenta

Abbreviations: AF, amniotic fluid; Ig, immunoglobulin; RT-PCR, real-time polymerase chain reaction; UCB, umbilical cord blood.
[a]If permission for fetal autopsy obtained.

whereas three did so for the first and second trimester.[28,30,110] Not all samples described in Table 3 carry equal weight in proving vertical transmission. Table 4 describes the sampling methods used to confirm MTCT. Reliable viral isolation from placenta, amniotic fluid and fetal blood or tissues would constitute strong evidence and provide indisputable proof of intrauterine infection.

The majority of studies collected neonatal airway swabs for SARS-CoV-2 RT-PCR to test for transplacental infection. Paired maternal-perinatal testing, that is, with vaginal, cervical or rectal swabs was performed to detect genital tract viral shedding during vaginal delivery in 22.5% of case series.[28,75,76,80,81,85,90,96,101] Other samples included amniotic fluid (reported in 35.0% of publications), UCB (32.5%) and placenta (30.0%). Breast milk was also tested for SARS-Co-V-2 using RT-PCR in 22.5% of studies. Five studies reported IgG and IgM serology in the mother and neonate[75,77,80,103,105] with additional cytokine assays.[77,104] Four studies evaluated the placenta for infectious pathology.[27,29,30,54,80,91] Twenty-five percentage of these reports documented neonatal SARS-CoV-2 infection.[75,77,94,100-105,109] One case series described positive neonatal RT-PCR at 36 hours postpartum although amniotic fluid RT-PCR was negative.[100] Reports from Italy, Peru and Iran attributed neonatal infection to horizontal transmission from RT-PCR positive mothers via breastfeeding without masks (respiratory droplets) and vaginal delivery (exposure to genital secretions)[101-103] due to maternal seroconversion a few days postpartum. A total of 57 amniotic fluid samples were tested, and only one sample (1.8%) tested positive for SARS-CoV-2 virus by RT-PCR. In this preterm neonate, amniotic fluid tested positive on RT-PCR, but UCB, neonatal nasal and throat swabs, which were negative at birth, only

became positive 24 hours postpartum.[101] Two reports suggested possible transplacental infection due to raised neonatal IgM antibodies and abnormal cytokine levels after birth, as IgM is not trafficked transplacentally and may have been formed by the fetus in response to in utero exposure to SARS-CoV-2.[75,77] In both reports, RT-PCR of neonatal nasopharyngeal swab and maternal vaginal secretions were negative.

These studies have several limitations in addressing the issue of MTCT. Most report infections in the third trimester where the duration between infection and delivery is relatively short. The risks of transplacental infection in the first and second trimesters, with a potentially longer fetal exposure time, are unclear. Although most patients were delivered by caesarean section, the evidence does not suggest a clear association between neonatal COVID-19 and vaginal delivery, except in one case.[93,102] Given increasing evidence of asymptomatic carriage amongst pregnant women, larger prospective studies are required to determine the risk of transmission during vaginal delivery, and whether this differs between asymptomatic and symptomatic pregnant women.[111,112]

Is there evidence of SARS-CoV-2 transplacental infection?

IgM assays are prone to technical errors.[113] Uncertainty remains over the likelihood and possible mechanisms of MTCT for SARS-CoV-2. Therefore, seropositivity requires reflex testing, such as, virus neutralisation, IgG avidity index, molecular and immunoblotting.[114] Dong et al reported a sudden decrease in IgM levels barely above the positive threshold when the assay was repeated for a 2-week-old infant.[75] This rapid drop in SARS-CoV-2 IgM differs from serological trends observed in common congenital infections, where raised IgM may be detected for up to 19 months in ZIKV,[115] 3 months in CMV and 6 months in rubella.[116,117]

TABLE 3 Summary of testing for MTCT of SARS-CoV-2

| Author and country of origin | Number of subjects | Maternal diagnostic criteria | Mode of delivery | Gestational age (w:weeks, d:days) | Paired peri-partum maternal testing | | Peri-partum material | | | | Neonatal | | | Breast milk | Possible evidence of transplacental infection |
					Vaginal / cervical	Rectal	AF	Placenta	Cord Blood	Other peri-partum material	Airway	Serum IgM/IgG	Other		
Yang P[79] (PRC)	7	A and B	7 CS	36w0d to 38w2d			√		√		√				No
Xiong X[80] (PRC)	1	A and B	1 VD	38w4d	√		√			Placental histology	√	√	Anal	√	No
Peng Z[81] (PRC)	1	A and B	1 CS	35w3d	√		√	√	√		√		Anal, serum, urine	√	No
Lee DH[82] (Korea)	1	A and B	1 CS	37w6d	√		√	√	√		√				No
Fan C[83] (PRC)	2	A and B	2 CS	36w to 37w	√		√	√	√		√			√	No
Chen H[70] (PRC)	9	A and B	9 CS	36w0d to 39w4d			√		√		√			√	No
Chen R[84] (PRC)	17	RT-PCR Throat Swab	17 CS	Third trimester							√				No
Dong L[75] (PRC)	1	A, B and C	1 VD	34w2d	√						√	√	CT thorax	√	Raised neonatal IgM and IgG but rest of tests inc. neonatal CT thorax negative. Asymptomatic.
Zeng H[77] (PRC)	6	A, B and C	6 CS	Third trimester							√	√	Blood		Two neonates with raised IgM and IgG. Three neonates with only raised IgG. Rest of tests negative. All neonates asymptomatic.
Li Y[85] (PRC)	1	A	1 CS	35w2d		Stool	√	√	√		√		Blood, stool, urine	√	No
Zhu H[86] (PRC)	9 (10 neonates, 1 twin)	A and B (2 had negative throat RT-PCR)	7 CS, 2 VD	34w5d to 39w0d							√				Negative swabs. Two neonates had thrombocytopaenia and transaminitis. One died.
Chen S[87] (PRC)	5	A and B	2 CS, 3VD	38w6d to 40w4dw			√	√	√		√				No
Zambrano[88] (Honduras)	1	A	1 VD	32w							√		Blood		No
Wang X[89] (PRC)	1	A and B	1 CS	30w			√	√	√		√		Gastric juice, stool	√	No
Gidlof[90] (Sweden)	1 (twin preg)	A	1 CS	36w2d	√						√			√	No
Chen S[91] (PRC)	3	A	3 CS	Third trimester				√		Placental histology	√				No Histology normal.
Liu W[92] (PRC)	19	A for 10 9 diagnosed clinically	18 CS, 1 VD	35w2d to 41w2d			√		√		√		Blood, urine, stool	√ for 10	No

TABLE 3 (Continued)

Author and country of origin	Number of subjects	Maternal diagnostic criteria	Mode of delivery	Gestational age (w:weeks, d:days)	Paired peri-partum maternal testing Vaginal/cervical	Rectal	AF	Placenta	Cord Blood	Other peri-partum material	Airway	Serum IgM/IgG	Other	Breast milk	Possible evidence of transplacental infection
Liao J[93] (PRC)	10	B	10 VD	36w2d to 40w2d							√				No
Khan S[94] (PRC)	17	A and / or B	17 CS	35w5d to 41w0d					√		√				Two neonate nasopharyngeal swabs positive with one developing neonatal pneumonia, four others had neonatal pneumonia with negative nasopharyngeal swabs.
Lu D[95] (PRC)	1	A and B	1 CS	38w							√		Blood		No
Lei D[96] (PRC)	9 (4 delivered)	B	1 TOP 3 CS, 1 VD, 4 ongoing	2 term and 2 premature	√		√		√		√			√	No
Qiancheng X[97] (PRC)	22 delivered, 24 neonates (2 twins)	A or C	17 CS, 5 VD, 2 ongoing, 4 TOP	Third trimester							√				No
Chen Y[98] (PRC)	4	A and B	3 CS, 1 VD	37w2d to 38w4d							√ Done on 3 neonates				No
Li N[99] (PRC)	34 (16 confirmed, 18 suspected)	A and / or B	30 CS, 4 VD	33w6d to 40w4d							√				No
Hu X[100] (PRC)	7	A and B	6 CS, 1 VD	38w2d to 41w2d				√			√		Blood, urine, stool		One neonate nasopharyngeal swab positive 36 h after birth.
Zamaniyan M[101] (Iran)	1	A and B	1 CS	32w	√		√	√	√		√				AF positive. Neonate nasopharyngeal swab positive 24 hours after birth.
Ferrazzi E[102] (Italy)	42	A	18 CS, 24 VD	Third trimester							√				Five positive. Two at 24 h but allowed skin-to-skin contact and breastfeeding without mask. Two more >36 h after delivery.
Alzamora MC[103] (Peru)	1	A, B and C	1 CS	33w0d							√	√			Positive neonatal nasopharyngeal swab. Serology negative.

TABLE 3 (Continued)

Author and country of origin	Number of subjects	Maternal diagnostic criteria	Mode of delivery	Gestational age (w:weeks, d:days)	Paired peri-partum maternal testing		Peri-partum material				Neonatal			Breast milk	Possible evidence of transplacental infection
					Vaginal/cervical	Rectal	AF	Placenta	Cord Blood	Other peri-partum material	Airway	Serum IgM/IgG	Other		
Zeng L[104] (PRC)	33	A	26 CS, 3 VD	Third trimester							√		Anal		Three neonates nasopharyngeal and anal swabs positive from 48 h.
Buonsenso D[105] (Italy)	7 (2 deliveries)	Throat swab RT-PCR	2 CS	35w5d to 38w3d				√	√		√	√	Rectal		Late onset neonatal COVID-19 for one neonate. Nasopharyngeal and anal swab positive at 15 d after birth. Maternal contact present.
Kirtsman M[106] (Canada)	1	A	CS	35w5d				√			√		Stool, plasma		Around the chorionic villi these is diffuse early infarction and crowding of inflammatory cells. Nasopharyngeal swab positive on day of birth onwards. Plasma positive from fourth day of life and stool positive after 1 week of life.
Kuhrt K[107] (UK)	1 (MCDA twins)	A	CS	32w6d							√				Placental histology – mild underperfusion and rapid villous maturation, may be due to abruption and/or hypoxic changes in the placenta.
Govind A[108] (UK)	9	A	8 CS, 2 VD	27 to 39w							√				All negative.
Sun M[109] (PRC)	3	A	CS	30w5d to 37w0d							√		CT thorax		One neonate positive nasopharyngeal swabs at Day 6. Another neonate with CT scan findings and clinical features suggestive at Day 6.
Baud D[28] (Switzerland)	1	A	Miscarriage	19w	√		√	√ (Submemembrane and cotyledon)	√	Placental histology	√		Anus, liver, thymus, lung, armpit		Funisitis at umbilical cord.

(Continues)

TABLE 3 (Continued)

Author and country of origin	Number of subjects	Maternal diagnostic criteria	Mode of delivery	Gestational age (w:weeks, d:days)	Paired peri-partum maternal testing		Peri-partum material				Neonatal			Breast milk	Possible evidence of transplacental infection
					Vaginal / cervical	Rectal	AF	Placenta	Cord Blood	Other peri-partum material	Airway	Serum IgM/IgG Other			
Yu N[110] (China)	2	Not stated	Amniocentesis	Amniocentesis done at 16w and 17w			√								AF negative for RT-PCR and IgM/IgG.
Penfield CA[54] (USA)	32	Not stated	Placental evaluation	27w5d to 41w3d			(Submembrane and amniotic surface	√			√				Three placental/membrane swabs positive.
Baergen RN[27] (USA)	20	Not stated	Placental evaluation	32w2d to 40w4d						Placental histology					10 cases showed fetal vascular malperfusion or fetal vascular thrombosis.
Hosier H[30] (USA)	1	A	Placental evaluation	22w				√		Umbilical cord		Heart and lung			Placental histology – extensive inflammatory infiltrate (T-lymphocytes, macrophages) and widespread perivillous fibrin. Viral particles observed in placental cell cytoplasm on electron microscopy.
Algarroba GN[29] USA)	1	Not stated	Placental evaluation	28w4d				√							Maternal surface contained areas of vasculopathy. Matured chorionic edematous villi. Viral particle visible invading syncytiotrophoblast.

Abbreviations: A, Maternal oral or nasopharyngeal RT-PCR; Ab, antibody; AF, amniotic fluid; B, Chest computed tomography; C, SARS-CoV-2 maternal serum IgG and IgM; CS, caesarean section; CT, Computed tomography; CXR, chest x-ray; Ig, immunoglobulin; MCDA, monochorionic diamniotic; MTCT, mother-to-child-transmission; PRC, People's Republic of China; RT-PCR, real-time polymerase chain reaction; TOP, termination of pregnancy; UCB umbilical cord blood; VD, normal vaginal delivery.

TABLE 4 Samples used in various studies (n = 40) to confirm or exclude MTCT

Test for MTCT	Number of studies using the tests	Percentage
Neonatal airway swabs RT-PCR	36	90.0
Other neonatal tissues (anal swab/rectal swab/urine/faeces/blood/gastric juice)	12	30.0
Neonatal computed tomography of chest	2	5.0
Amniotic fluid RT-PCR	14	35.0
Umbilical cord blood RT-PCR	13	32.5
Placenta RT-PCR	12	30.0
Breast milk RT-PCR	9	22.5
Peri-partum vaginal or cervical secretions RT-PCR	8	20.0
Peri-partum maternal rectal swab/stool RT-PCR	3	7.5
Serum immunoglobulins IgM and IgG	5	12.5
Placental/cord histology	5	12.5

Abbreviations: Ig, immunoglobulin; MTCT, mother-to-child-transmission; RT-PCR: real-time polymerase chain reaction.

Plausible reasons for antigen-specific IgM in the fetus may be attributed to inflammation at maternal-fetal interface (maternal hypoxic injury or viral cytotoxic effects) that may cause leakages of maternal IgM. Additionally, ascending infections from the lower genital tract may facilitate transfer of maternal immunoglobulins across the placental barrier, as inferred from the 4-fold increase in placental IgM trafficking in the presence of moderate infection.[63] Conclusions on transplacental infection cannot be based solely on the presence of neonatal IgM, as its significance with concomitant negative neonatal RT-PCR remains unclear. FDA guidelines on serological testing generally support its use to confirm past exposure, but not to diagnose COVID-19. To date, not all commercially available serological assays have been validated by the FDA.

Histological evaluations of the placenta and umbilical cord shed further light on the maternal-fetal response to viral infections.[28] Placental trophoblastic apoptosis and vascular damage contribute to increased permeability of the placenta.[118] In SARS-CoV-1, during the acute phase, maternal hypoxia may have caused maternal placental blood flow disturbances leading to increased subchorionic or intervillous fibrin deposition; whilst during the convalescent phase, extensive fetal thrombotic vasculopathy with avascular fibrotic villi was observed. However, these changes probably result from pathophysiological responses to maternal respiratory disease rather than direct viral effects.[24,25] This acute respiratory distress is associated with 80% preterm delivery and 40% fetal growth restriction rates in a cohort of five cases of second and third trimester infections.[24] Recently, more studies have reported placental histology in SARS-CoV-2 infections. Although an earlier examination of three placentas in the third trimester from women with mild COVID-19 symptoms did not show significant abnormalities,[91] larger case series now show evidence of maternal vascular malperfusion and chronic inflammatory pathology.[27,119] The placenta from the one patient with acute hypoxia due to COVID-19 pneumonia showed signs of ascending acute chorioamnionitis and funisitis. However, 20%-73% of placentas had maternal vascular malperfusion and 13%-20% had chronic inflammatory pathology. These occurred in women with mild COVID-19. Other causes for these changes that should be considered include hypercoagulopathy associated with COVID-19,[120] antiviral immune response[121,122] or possible endotheliitis.[123] Placentas from second trimester miscarriages had funisitis, villous edema and retroplacental haematoma but in a context of maternal normoxia.[28,119] Placental inflammation may contribute to disruption of the maternal-placental interface and can potentially facilitate transplacental infection. The COVID-19 symptoms and histopathological changes in the placenta do not increase the risk of fetal growth restriction but rather increases preterm births.[26] It is therefore important to understand the immune defence mechanisms at the maternal-fetal interface that prevent transplacental transmission of SARS-CoV-2.

3.2 | Does the ACE-2 receptor play a role in MTCT?

Multiple reports have confirmed that SARS-CoV-2 gains entry into cells utilising the ACE-2 receptor and the serine protease TMPRSS2 for S protein priming.[124-126] In humans, ACE-2 mRNA gene and receptor protein are highly abundant in the early placenta, especially in SCT and villous stroma.[127] Single-cell RNA sequencing (scRNA-seq) revealed that the ACE-2 gene is highly expressed in human stromal and perivascular decidual cells, VCT and SCT. In early pregnancy, there is expression of TMPRSS2 genes in VCT and low levels in SCT. Low to negligible levels were observed in EVT at 8 weeks' gestation with increasing expression observed at 24 weeks.[128,129]

In contrast to other studies, Zheng et al reported low RNA expression of ACE-2 gene in all maternal-fetal interface cells derived from first trimester decidua and placentas.[130] Compared with all the interface cells, decidual perivascular cells (PV1 cluster) had relatively higher ACE-2 RNA.[130] Although *ACE-2* gene expression in SCT, VCT and PV1 cells was still demonstrable, *TMPRSS2* gene was undetectable by scRNA-seq.[131] PV1 cells only expressed 8 of the 35 viral process-related genes expressed in type II alveolar cells. Therefore, SARS-CoV-2 has fewer means of entering PV1 cells at the maternal-fetal interface and thus would be less likely to cause transplacental infection. However, Hou et al has recently reported that the single-cell (cytospin) RNA-in situ hybridization (ISH) technique is 5-10 times more sensitive in ascertaining cell-type-specific expression patterns compared to scRNA-seq. scRNA-ISH showed a gradient of SARS-CoV-2 infection that was highly correlated to the expression of ACE-2 from the upper to lower respiratory tract[132] Therefore, the actual spatial-temporal expression of ACE-2 and TMPRSS2 or other viral entry-related genes within the cell populations at the maternal-fetal interface still awaits further scrutiny.

Analysis of TMPRSS2 transcriptome abundance across the HPA RNA-seq and FANTOM5 CAGE datasets demonstrates a paucity of RNA translation in the placenta [eg, 0.3 pTPM (protein-coding transcripts per million) vs 86.5 pTPM in the lung], and undetectable protein expression by immunocytochemistry.[133,134] The relative absence of TMPRSS2 may partly explain the scarcity of viral particles in placenta on electron microscopy[29,30] which stands in contrast to the abundance of virus in the kidneys[135] and may be the key reason as to why we have not seen conclusive evidence of MTCT in COVID-19. As co-expression of proteins ACE-2 and TMPRSS2 are crucial for SARS-CoV-2 gaining cytoplasmic entry, and the absence of one or both proteins from the cell membrane inhibits transplacental infection.

3.3 | Breastfeeding – another potential route of transmission

Another potential route for SARS-CoV-2 transmission to the newborn is via breast milk. Comparing scRNA-seq datasets extracted from The Cancer Genome Atlas and FANTOM5, it was observed that ACE-2 translation in breast tissue was similar to that of the lung tissue.[136] Even though the ACE-2 receptor is expressed in breast tissue, most reports have not detected SARS-CoV-2 in breast milk.[70,75,76,81] In one report, a sample from one patient was transiently positive for SARS-CoV-2 on the first day of collection but subsequently tested negative 2 days later.[137] Although the various guidelines have endorsed the relative safety of breastfeeding whilst infected with COVID-19, more data on breastfeeding safety is still needed.[138,139]

3.4 | How does SARS-CoV-2 affect early pregnancies?

The PRIORITY (Pregnancy CoRonavIrus Outcomes RegIsTrY) study spearheaded by University of California, San Francisco[140] and COVI-PREG in Lausanne, Switzerland[141] are currently recruiting pregnant and recently pregnant women with known or suspected COVID-19 disease to understand the clinical course of the disease and risks of complications such as miscarriage, stillbirth, preterm labour and neonatal health. A recent meta-analysis on pregnancy and perinatal outcomes of SARS-CoV-2 infection has reported higher rates of preterm births, miscarriage, preeclampsia, caesarean deliveries and perinatal deaths compared to the general population.[26] Although reports of deliveries in third trimester COVID-19 women continue to accumulate, reports on earlier trimesters remain scant. A recently published correspondence reported that second trimester pregnancy amniotic fluid obtained from two women who had COVID-19 disease tested negative for SARS-CoV-2 and had normal levels of IgG and IgM. It is not known if there is an ideal time window for an amniocentesis to maximise diagnostic yield as with CMV.[142]

4 | CONCLUSION

The co-expression of ACE-2 receptor and TMPRSS2 protease required for SARS-CoV-2 to gain cytoplasmic entry is not present in cells at the maternal-fetal interface. This corroborates available clinical data based on systematically assessed maternal and perinatal samples, which have not demonstrated consistent and conclusive features of MTCT of SARS-CoV-2 in pregnancy in ways typically seen with classical congenital infections. There is clear evidence, however, that there are histopathological changes in the placenta from women infected with even mild COVID-19 showing maternal vascular malperfusion and inflammatory changes, through mechanisms that need further elucidation. It is unclear if this can disrupt the maternal-placental interface and permit transplacental transmission of SARS-CoV-2 virion, although this is unlikely to be a frequent occurrence. For cases in which vertical transmission is confirmed or suspected, larger, longer term studies with longitudinal follow-up into childhood are needed to establish the full implications of these findings. Reliable viral isolation from placenta and fetal blood or tissues are required to provide indisputable proof of intrauterine infection, and the underlying mechanism of insult, direct or molecular, should be established. In the absence of these, the detection of viral nucleic acids on amniocentesis would provide strong evidence.[57] Aborted products of conception (POC), including decidua, should be analysed for SARS-CoV-2, although isolation of viral nucleic acids here may represent early decidual infection or contamination by vaginal fluid, not necessarily transplacental transmission. Fetal and extra-fetal tissues should be examined for ACE-2 expression, and the maternal-infant dyad tested for SARS-CoV-2 viral nucleic acids by RT-PCR. Estimates suggest that the COVID-19 pandemic may last until 2022.[143] Until the many uncertainties regarding MTCT remain unanswered, social distancing,[144] universal testing in high prevalence areas[145] and proper intrapartum infection control[71] will remain mainstays in reducing transmission of SARS-CoV-2 from mother to baby. Separation of infant from mother is controversial and not universally recommended; therefore, obtaining conclusive evidence on diagnostic performance of various newborn samples poses a challenge. Biological samples as detailed in Table 2 should be considered with special consideration to perform repeated neonatal airway RT-PCR every 2 to 3 days postnatal if the initial swab is negative given reports of late neonatal infections.[105]

CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

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