

Mechanisms of SARS-CoV-2 Placental Transmission

Karol Gostomczyk^{1,2}✉ · Jędrzej Borowczak¹ · Marta Siekielska-Domanowska¹ · Krzysztof Szczerbowski¹ · Mateusz Maniewski¹ · Mariusz Dubiel¹ · Łukasz Szyłberg^{1,2,3} · Magdalena Bodnar^{1,3}

Abstract

The widespread occurrence of SARS-CoV-2 infections and the diverse range of symptoms have placed significant strain on healthcare systems worldwide. Pregnancy has also been affected by COVID-19, with an increased risk of complications and unfavorable outcomes for expectant mothers. Multiple studies indicate that SARS-CoV-2 can infiltrate the placenta, breach its protective barrier, and infect the fetus. Although the precise mechanisms of intrauterine transmission remain unclear, factors such as perinatal infection, macrophages, sexual intercourse, and the virus' interaction with host angiotensin-converting enzyme 2 (ACE2) and neuropilin-1 (NRP-1) proteins appear to play a role in this process. The integrity of the placental barrier fluctuates throughout pregnancy and appears to influence the likelihood of fetal transmission. The expression of placental cell receptors, like ACE2, changes during pregnancy and in response to placental damage. However, due to the consistent presence of others, such as NRP-1, SARS-CoV-2 may potentially enter the fetus at different stages of pregnancy. NRP-1 is also found in macrophages, implicating maternal macrophages and Hofbauer cells as potential routes for viral transmission. Our current understanding of SARS-CoV-2's vertical transmission pathways remains limited. Some researchers question the ACE2-associated transmission model due to the relatively low expression of ACE2 in the placenta. Existing studies investigating perinatal transmission and the impact of sexual intercourse have either involved small sample sizes or lacked statistical significance. This review aims to explore the current state of knowledge regarding the potential mechanisms of COVID-19 vertical transmission, identifying areas where further research is needed to fill the gaps in our understanding.

Keywords

SARS-CoV-2 · Vertical transmission · Mechanisms · ACE2 · TMPRSS2 · NRP-1

Received: 30 June 2023 / Accepted: 7 September 2023/

© L. Hirszfeld Institute of Immunology and Experimental Therapy, Wrocław, Poland 2023

1. Introduction

The COVID-19 pandemic caused by the SARS-CoV-2 virus compromised the functioning of health systems in many countries worldwide. SARS-CoV-2 was discovered in December 2019 and described as a positive, single-stranded RNA virus that causes severe pneumonia of unknown etiology (Gorbalenya et al., 2020; Zhou et al., 2020). Due to the virus' transmission mechanism and lack of characteristic symptoms, the attempts to control the pandemic were only partially successful. The high mutagenic potential of SARS-CoV-2 causes the emergence of new viral strains. Since the disease course and symptoms are constantly evolving, disease monitoring and treatment continue to pose clinical difficulties (European Centre for Disease Prevention and Control, 2023).

Over the last years, several mechanisms of SARS-CoV-2 infection have been proposed. Presently, binding the host angiotensin-converting enzyme 2 (ACE2) receptors by

the viral S protein is considered the main route of SARS-CoV-2 cell entry. The enzyme is highly expressed in the kidneys, cardiovascular system, respiratory epithelium, and placenta; thus, the spectrum of coronavirus possible complications is broad (Salamanna et al., 2020; Zaim et al., 2020). Undifferentiated airway epithelia highly expressing ACE2 seem particularly susceptible to coronavirus infection, and the levels of ACE2 in lungs increase during severe SARS-CoV-2 infection (Jia et al., 2005; Gheware et al., 2022). The widespread expression of ACE2 in the human body seems to reflect the broad scope of clinical manifestation of the disease. Nevertheless, the complicated net of viral–host protein interactions allowed us to identify other potential mechanisms of disease transmission, many of which are still not fully investigated (Jackson et al., 2022).

The SARS-CoV-2 pandemic has also impacted pregnancy course and clinical outcomes, contributing to increased maternal mortality and morbidity (Slomski, 2022). Since pregnant women are prone to viral infection and even asymptomatic infections during pregnancy may affect fetal development, understanding the routes of fetal infections seems necessary for early monitoring and treatment (Gychka et al., 2022). The etiology of fetal injury is not well-defined, but many studies suggested that SARS-CoV-2 can be transmitted vertically from the mother to the developing fetus (Kotlyar et al., 2021).

¹ Department of Obstetrics, Gynaecology and Oncology, Chair of Pathomorphology and Clinical Placentology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń, Poland

² Department of Tumor Pathology and Pathomorphology, Oncology Centre – Prof. Franciszek Łukaszczyk Memorial Hospital, Bydgoszcz, Poland

³ Chair of Pathology, Dr. Jan Biziel Memorial University Hospital No. 2, Bydgoszcz, Poland

✉ karolgostomczyk.research@gmail.com

The infection seems to occur prenatally since even the immediate isolation of a newborn from a COVID-19-positive mother does not always prevent viral transmission (Alzamora et al., 2020). Originally, SARS-CoV-2 transmission via the placenta was considered unlikely, but recent studies suggest that the virus impairs the integrity of the maternal–fetal barrier and enters fetal tissues (Pettiroso et al., 2020). The mechanisms of intrauterine transmission remain unclear, although some possible routes exist.

This review presents and discusses the current state-of-the-art on the possible mechanisms of COVID-19 vertical transmission to define gaps in our knowledge that may be addressed in further studies.

2. Methods and Selection Criteria

2.1. Methods

For this review, we searched multiple databases, including PubMed, Google Scholar, WHO COVID-19 database, China National Knowledge Infrastructure (CNKI), American Congress of Obstetricians and Gynecologists (ACOG) indications, and Centers for Disease Control and Prevention (CDC) database. The search terms included: “COVID-19”, “SARS-CoV-2”, “Vertical transmission”, “Severe acute respiratory syndrome coronavirus”, “Pregnancy”, “Placenta”, “Neuropilin-1”, “ACE2”, “TMPRSS2”, “Macrophages”, “Placentitis”, and “Cytokine storm”. We identified the article titles containing the keywords on PubMed and Google Scholar.

2.2. Inclusion criteria

All the included research were selected and assessed only if they met the following criteria:

- (1) positive maternal SARS-CoV-2 infection confirmed by PCR test;
- (2) reporting of neonatal outcome;
- (3) studies published in English with indicated dates and locations and indexed in MEDLINE;
- (4) studies that include vertical transmission of SARS-CoV-2 infection during pregnancy;
- (5) studies on changes and correlations that SARS-CoV-2 provokes in the body; and
- (6) studies including inflammatory changes, cytokines, and cells in SARS-CoV-2 infection during pregnancy.

2.3. Exclusion criteria

Studies excluded in this review were letters to the editor, abstracts without full text, clinical trials, observational studies,

conference proceedings, and studies published before the COVID-19 pandemic.

3. COVID-19 Infection of the Placenta

3.1. The mechanisms of placental injury related to SARS-CoV-2 infection

The human placenta acts as a physical and immunological barrier that allows the semi-allogeneic fetus to develop inside the mother’s uterus and prevents microorganisms from affecting the fetus (Rackaityte and Halkias, 2020). The placental barrier consists of two main layers: stem-like, mono-nucleated cytotrophoblasts and syncytiotrophoblasts, which consist of fused cytotrophoblasts (Kapila and Khalid, 2023). During syncytialization, cytotrophoblasts fuse with the overlying syncytiotrophoblasts, forming the placental surface. Cytoplasmic protrusions from cytotrophoblasts get through between syncytiotrophoblast cells, supplying them with nutrients and organelles (Baergen et al., 2022). Hence, the latter are more resistant to pathogens.

High viral load and immunocompromising pathogens can compromise the placental defense systems and enable fetal transmission. Furthermore, constant interactions between the outer placenta layer and maternal blood damage the syncytiotrophoblasts, making them prone to infections (Robbins and Bakardjiev, 2012; Megli and Coyne, 2022; Kapila and Khalid, 2023). Since the syncytiotrophoblasts lack intercellular gap junctions, pathogens can also cross the barrier through leukocytes (Celik et al., 2020).

The tightness of the placental barrier varies in different stages of pregnancy. Virions in the maternal bloodstream enter the fetus near the extravillous trophoblast’s cells in decidua or where syncytiotrophoblasts contact maternal blood. The passage is easier in the early stage when cells are yet to fully fuse into syncytiotrophoblasts and before delivery when the syncytium begins to deteriorate (Robbins and Bakardjiev, 2012). The studies reporting SARS-CoV-2 infections among pregnant women reflect the histopathological changes in the placenta (Mirbeyk et al., 2021). The physiological immunomodulation allows normal fetal development but also seems to be the leading cause of why pregnant women are most prone to infection and its severe course in the third trimester of pregnancy. Similarly, only infections in the third trimester, particularly after the 34th week of pregnancy, were associated with an increased risk of preterm birth (Mirbeyk et al., 2021; Fallach et al., 2022). The infection is also associated with increased maternal and fetal death risk, preeclampsia, low newborn body mass, and lower appearance, pulse, grimace, activity, and respiration scores (APGAR) (Pathirathna et al., 2022).

3.2. Changes in placenta caused by SARS-CoV-2 infection

Although the increased looseness of the placental barrier may facilitate fetal infection, SARS-CoV-2 vertical transmission is rare (Garcia-Flores et al., 2022). However, even sporadic transmission poses a significant health concern due to physiological immunosuppression and insufficient transfer of the anti-SARS-CoV-2 antibodies from the mother to the fetus (Edlow et al., 2020). In Mirbeyk et al.'s (2021) study, only 5% of nasopharyngeal samples collected from newborns of SARS-CoV-2-positive mothers were positive.

Aside from the transmission route, other factors contributing to perinatal complications have been identified. For example, COVID-19 increases the risk of venous thrombosis, which Ahmed et al. (2020) recently explained concerning Virchow's triad. Its elements, arising from vascular remodeling, are commonly found in the placentas of COVID-19-positive mothers. They disturb the blood flow in the placenta, increase vascular resistance, and can induce a compensatory increase in local blood pressure that results in vascular injury. Decreased lumen area of placental arteries and artery wall thickening occur in SARS-CoV-2-positive women independent of symptoms. The overlap of vessel wall abnormalities, blood flow abnormalities, and hypercoagulable state increases the risk of thrombosis while gradually diminishing placental functions (Khalil and Granger, 2002; Xu et al., 2020).

SARS-CoV-2 damages the placenta by inducing inflammation in the villous chamber (Bouachba et al., 2021). However, the scope of pathological changes differs significantly between stillbirth and live birth placentas in SARS-CoV-2-positive women. Konstantinidou et al. (2022) found that over 75% of the maternal intervillous space in the stillbirth placentas was obliterated, while similar changes are much rarer in live-born neonates. SARS-CoV-2-infected placentas did not differ macroscopically from their healthy equivalents but showed signs of chronic lymphoplasmacytic deciduous, villous fibrosis, capillary injury, and blood extravasation fetal vessels thrombosis (Table 1). These changes are consistent with fetal vascular malperfusion (FVM) – placental lesions that indicate abnormal perfusion of the fetal villous parenchyma, usually caused by umbilical cord obstruction (Baston-Buest et al., 2011). Features characteristic of maternal vascular malperfusion (MVM), abnormal or damaged maternal vessels, and intervillous thrombus were also more common in SARS-CoV-2-infected placenta than in their healthy counterparts (Shanes et al., 2020; Smithgall et al., 2020; Boyraz et al., 2022). MVM and FVM are among the most common histological changes in COVID-infected placentas and constitute one of the most important mechanisms by which COVID-19 affects fetal development (Hosier et al., 2020; Sharps et al., 2020). Schwarz et al. (2020) proposed criteria to standardize the molecular identification of the virus on the fetal side of the

Table 1. Pathologies and clinical manifestations

Level of changes	Pathology	Clinical manifestation
Placental	Venous thrombosis Hemodynamic changes Hypercoagulability Chronic histiocytic intervillitis Syncytiotrophoblast necrosis Fetal and maternal VM Massive perivillous fibrin deposition Lymphoplasmacytic deciduous Extravasation of erythrocytes Thrombosis of placental vessels Cholangitis	Miscarriage Stillbirth Fetal growth restriction Early preeclampsia Neurosensory development delay CNS disorders FIRS
Systemic	Cytokine storm Venous thrombosis Hemodynamic changes Hypercoagulability	

CNS, central nervous system; FIRS, fetal inflammatory response syndrome; VM, vascular malperfusion.

placenta. Since then, the transmission must be confirmed by detecting the viral antigens by immunohistochemistry or viral nucleic acid by RNA scope methods. SARS-CoV-2 placentitis is defined by the coexistence of histiocytic intervillitis, perivillous fibrin deposition, and trophoblast necrosis, regardless of whether the transplacental transmission was confirmed (Watkins et al., 2021). Severe cases may be associated with positive immunostaining for SARS-CoV-2 spike protein and positive reverse-transcription polymerase chain reaction test of placental tissues (Konstantinidou et al., 2022). Aside from confirming viral RNA in the placenta, its detection in the umbilical cord also suggests diagnosis (Menter et al., 2021). Given the sporadic nature of SARS-CoV-2 vertical transmission, the morphological changes in placentas from infected newborns are yet to be systematized. Furthermore, the transmission can be asymptomatic despite histopathological changes in the placenta – such as intervillous fibrinoid depositions and intervillitis (Boncompagni et al., 2022). On the other hand, SARS-CoV-2 infection during the third trimester of pregnancy correlates with high neonatal viremia and inflammation, which could manifest as neurological symptoms, such as axial hypertonia and opisthotonos (Vivanti et al., 2020). Therefore, identifying the mechanisms of SARS-CoV-2 vertical transmission is of critical importance.

4. Mechanisms of COVID-19 Vertical Transmission

4.1. The mechanism of SARS-CoV-2 cell entry

The SARS-CoV-2 virus consists of structural, such as the spike, envelope, nucleocapsid, and membrane, and non-structural proteins. Their main function is to facilitate viral infection and multiplication (Seyed Hosseini et al., 2020; Rojas-Rueda and Morales-Zamora, 2021). The virus uses

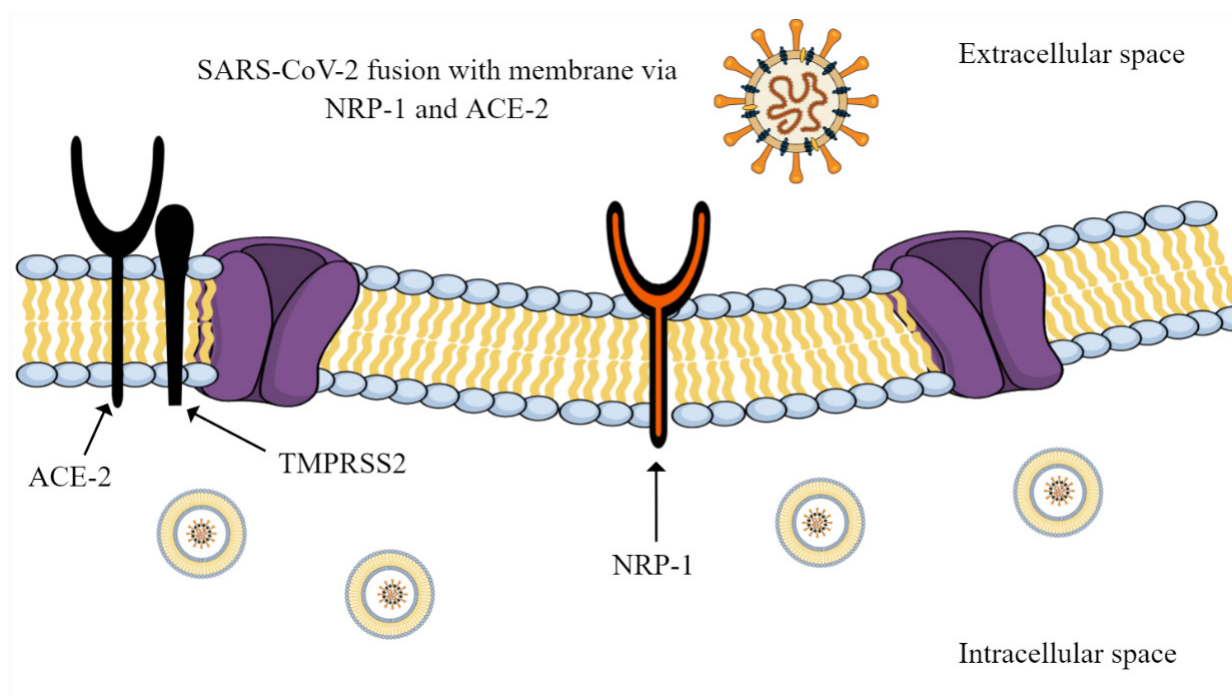


Fig 1. SARS-CoV-2 cell entry via ACE2 and NRP-1 receptors. ACE2, angiotensin-converting enzyme 2; NRP-1, neuropilin-1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2.

the receptor-binding domain of the spike S1 protein and receptor-binding motif (RBM) of the outer surface of the ACE2 and enters the host (Figure 1). ACE2 degrades angiotensin II, inhibiting the renin–angiotensin–aldosterone pathway (Glowacka et al., 2011; Samavati and Uhal, 2020). The host protease, TMPRSS, cleaves the S protein and enables viral cell entry (Glowacka et al., 2011). After entering the host cell, the virus replicates and synthesizes its structural proteins. SARS-CoV-2 infection of the placenta can be confirmed by immunostaining for spike protein of placental tissues, and its levels are high in severe cases of COVID-19. However, the replications of SARS-CoV-2 appear to be restricted in the human placenta (Takada et al., 2022). Two spots for virus binding and mutations at the RBM occur near the spots of ACE2 and determine the scope of host infection, playing an important role in the virus mutagenicity (Chlamydas et al., 2020; Samavati and Uhal, 2020).

4.2. ACE2 and TMPRSS2 protease

Various cells and tissues express ACE2, including syncytial trophoblasts, cytotrophoblasts, endothelial cells, decidual cells, and vascular smooth muscle of the villi (Nobrega Cruz et al., 2021). Co-expression of both ACE2 and TMPRSS2 was considered to be the main SARS-CoV-2 entry route. The placenta is a barrier against viral and bacterial infections but seems to lack efficacy in preventing COVID-19 vertical transmission (Mao et al., 2022).

Within the placenta, some tissues express both ACE2 and TMPRSS2 (Karuppan et al., 2021; Senapati et al., 2021). In the early gestation stages, co-expression of ACE2 and TMPRSS2 is high, and both proteins can be found in decidual areas (Hosier et al., 2020). As the gestational duration increases, the co-expression of ACE2 and TMPRSS2 decreases, resulting in a lower risk of viral transmission (Jing et al., 2020).

ACE2 levels are higher in the placenta than in the lungs, but their levels change at various stages of pregnancy. They may be upregulated by the infection, i.e., due to local ischemia and placental malperfusion (Yu et al., 2016). Since ACE2 expression is the highest during early pregnancy, early syncytiotrophoblast is more susceptible to high viral load (Ruan et al., 2022). ACE2 is also expressed at all stages of pregnancy, constituting a possible transmission route. Interestingly, TMPRSS2 is absent on the syncytial surface, and even its stimulated expression does not allow SARS-CoV-2 entry into the term placenta (Colson et al., 2021).

4.3. Perinatal and sexual intercourse-based transmission

The expression of ACE2 and TMPRSS2 in the breast is yet to be extensively investigated, but available reports suggest that the risk of SARS-CoV-2 transmission with breast milk is very low (Edlow et al., 2020; Garcia-Flores et al., 2022). As such, the American College of Obstetricians and

Gynecologists recommendations encourage SARS-CoV-2-positive mothers to breastfeed (Centers for Disease Control and Prevention, 2023; The American College of Obstetricians and Gynecologists, 2023).

Hudak et al. (2023) reported that 2.2% of newborns of COVID-19-positive mothers tested positive for SARS-CoV-2. While this data suggests perinatal infection, vaginal upstream transmission, while theoretically possible, seems unlikely and vaginal delivery may be chosen as a delivery route (Fenizia et al., 2021). Interestingly, the SARS-CoV-2 virus has been recently found in semen; nevertheless, the likelihood of transmission during sexual intercourse appears extremely low (Gacci et al., 2021; Donders et al., 2022).

4.4. Neuropilin-1 (NRP-1)

NRP-1 is a transmembrane coreceptor of the tyrosine kinase receptor for vascular endothelial growth factor and semaphorin. NRP-1 contains an N-terminal extracellular domain consisting of A (a1–a2), B (b1–b2), and C subdomains. Its b1 subdomain is complementary with the spike proteins of SARS-CoV-2, and their binding facilitates the SARS-CoV-2 cell's entry (Abebe et al., 2021). NRP-1 is vital in angiogenesis, axon conductance, and signal transduction (Naidoo et al., 2022). Its activity drives cell survival, proliferation, differentiation, and migration (Guo and Vander Kooi, 2015). NRP-1 is highly expressed in the respiratory tract, olfactory epithelium, and placental tissues at every stage of gestation (Mayi et al., 2021; Huang et al., 2022). NRP-1 potentiates SARS-CoV-2 infectivity; the furin cleaves the S1 fragment of SARS-CoV-2's S protein and binds to the NRP-1 cell at the cell surface, constituting another entry route for COVID-19 into the host cells (Daly et al., 2020; Abebe et al., 2021). Daly et al. (2020) showed that blocking this binding with antibodies restricts viral infection. Interestingly, the expression of the NRP-1 is much higher in human lung tissue, olfactory epithelium, and the placenta than the expressions of ACE2 and TMPRSS2 (Karuppan et al., 2021). NRP-1 has also been shown to be expressed by immune cells, including placental macrophages and T cells, suggesting their potential role in viral spread (Abebe et al., 2021).

Since the expression of the NRP-1 among the placental tissues is high, it may be involved in the virus' vertical transmission. NRP-1 is mainly found in the decidual cells, intermediate trophoblast, and syncytiotrophoblast (Abebe et al., 2021). NRP-1 is also uniquely expressed on small syncytiotrophoblast extracellular vesicles (Roy et al., 2017). Furthermore, the NRP-1 presence on the placental structures, macrophages, and monocytes is constant through all gestation stages, increasing the probability of NRP-1 being the main route of SARS-CoV-2 vertical transmission (Baston-Buest et al., 2011) (Figure 1).

4.5. Macrophages and monocytes

Macrophages constitute the core of placental inflammatory infiltration during SARS-CoV-2 infection. They can be subdivided into distinct populations, the most important being Hofbauer cells, which derive from the fetus, and the decidual macrophages of maternal origin (Chambers et al., 2021; Mezouar et al., 2021). All of them can damage the syncytiotrophoblasts and disrupt the maternal–fetal barrier integrity, facilitating viral transmission (Schwartz et al., 2020; Mao et al., 2022). Term placenta appears to have a larger fetal than maternal population of macrophages, but the proportion is likely to change throughout pregnancy (Mezouar et al., 2019). Nevertheless, maternal macrophages seem to play a key role in SARS-CoV-2 pathogenesis. After virus uptake, macrophages switch their phenotype from the immunosuppressive M2 subtype toward the inflammatory M1 subtype and start secreting proinflammatory mediators that damage placental tissue (Yao et al., 2019; Fu et al., 2020). Furthermore, they can also transport the virus directly into the fetus (Percivalle et al., 2021).

ACE2 does not appear necessary for macrophage infection by SARS-CoV-2, but the virus does not replicate in ACE2-deficient cells (Labzin et al., 2023). On the contrary, ACE2 and CD16 overexpression is associated with enhanced viral uptake, macrophage replication, and proinflammatory immune response (Sefik et al., 2022). Infected macrophages and monocytes migrate through the organisms, crossing the maternal–fetal barrier and spreading the infection (Jafarzadeh et al., 2020). The expression of NRP-1 on macrophages is an easy target for COVID-19 infection because the NRP-1 mRNA appears to be the highest in macrophages. It further implicates that macrophages play a critical role in fetal infections (Huang et al., 2022). SARS-CoV-2 can also enter macrophages by binding its spike (S) protein to toll-like receptor 4, a type I transmembrane protein, to increase ACE2 expression and facilitate viral entry (Aboudounya and Heads, 2021).

Models of ACE2-independent viral transmission have also been proposed. SARS-CoV-2-specific antibodies can bind the virus surface to the macrophage surface IgG Fc receptor. After host cell entry, viral RNA is released and can modify endosomal signaling. This mechanism is known as antibody-dependent enhancement (Karthik et al., 2020; Wang et al., 2022). Inflammation potentiates placental damage and increases macrophage transmission and virus penetrability through the placental barrier. The susceptibility to SARS-CoV-2 infection appears to be associated with impaired trophoblast differentiation, proinflammatory and regulatory immune cell activation, enhanced placental immune response, and complement overactivation (Chen et al., 2022).

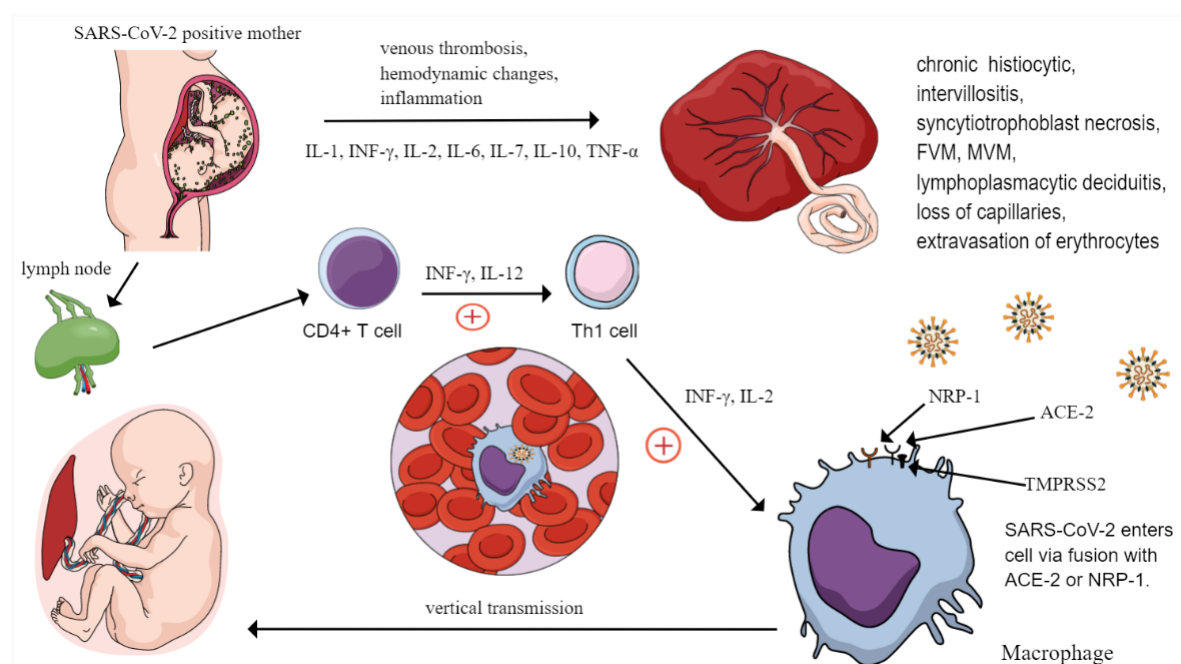


Fig 2. The role of macrophages in SARS-CoV-2 vertical transmission. ACE2, angiotensin-converting enzyme 2; FVM, fetal vascular malperfusion; IL, interleukin; INF- γ , interferon- γ ; MVM, maternal vascular malperfusion; NRP-1, neuropilin-1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2; TNF- α , tumor necrosis factor- α .

Table 2. The mechanisms and probability of SARS-CoV-2 transmission

Mechanism	Location in the body or transmission route	Transmission probability
ACE2 and TMPRSS2 protease	Heart, kidneys, testes, lungs, nasopharynx, smooth muscle cells, placenta	The risk of transmission is the highest during early pregnancy and decreases toward delivery
Perinatal transmission	Healthcare services and procedures	Extremely low risk of viral transmission
Breastfeeding	Breast milk	Extremely low risk of viral transmission
Sexual intercourse	Semen	Very low risk of viral transmission
NRP-1	Hofbauer cells, endothelial cells, smooth muscle cells, adipocytes, Sertoli cells, placenta	This is the most likely route of SARS-CoV-2 transmission
Macrophages and monocytes	Various tissues – especially placental macrophages	Very high risk of viral transmission

ACE2, angiotensin-converting enzyme 2; NRP-1, neuropilin-1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2.

During the maternal immune response to SARS-CoV-2 placentitis, CD163-positive M2-phenotype monocytes/macrophages and T cells migrate into the intervillous space, targeting mainly the syncytiotrophoblast. The infected maternal cells easily cross the placental barrier and may infect the fetus (Argueta et al., 2022). Considering the coexistence of multiple transmission routes, NRP-1 and ACE2 included, on the placenta and immune cells at every stage of pregnancy, macrophage-driven transmission seems probable (Figure 2). However, further trials are needed to verify this hypothesis.

Finally, SARS-CoV-2 can infect fetal histiocytes within the placental chorionic villus, called Hofbauer cells, causing syncytiotrophoblast injury (Reyes et al., 2017; Argueta et

al., 2022). In Schwartz et al.'s study (2021), SARS-CoV-2 infected Hofbauer cells in 18.2% (4/22) and syncytiotrophoblast in 95.5% (21/22) of examined placentas. The authors suggested that SARS-CoV-2 can extend beyond the trophoblast, and most SARS-CoV-2 transplacental fetal infections do not involve Hofbauer cells (Schwartz et al., 2021). However, even without invading villi or Hofbauer cells, fetal mononuclear cells and blood cells infected with SARS-CoV-2 seem to propagate placental infection and maternal-fetal transmission (Facchetti et al., 2020; Huang et al., 2020) (Table 2).

SARS-CoV-2 enters the cell by interacting with ACE2 and NRP-1. SARS-CoV-2's spike protein has two different domains; the first domain (S1) binds to the ACE2 receptors,

whereas the second (S2) interacts with the cell's membrane. The TMPRSS2 protease on the cell membrane cleaves the spike protein, enabling membrane fusion and viral entry. NRP-1's b1 subdomain binds with spike proteins of SARS-CoV-2, facilitating SARS-CoV-2 cell's entry.

SARS-CoV-2 infection activates the immune response and systemic inflammation, leading to hemodynamic changes and cytokine storm driven by interleukins, INF- γ , and TNF- α release. COVID-19 also induces INF- γ and IL-12-dependent differentiation of CD4⁺ T cells to Th1 cells in the lymph nodes, which then secrete INF- γ and IL-12, activating macrophages and cytotoxic T cells. Inflammation-associated placental injury, resulting mainly from chronic histiocytic intervillitis, can manifest as syncytiotrophoblast necrosis, FVM, MVM, lymphoplasmacytic deciduous, loss of capillaries, or extravasation of erythrocytes. These placental changes impair the integrity of the placental barrier, facilitating viral transmission. In such an environment, placental macrophages infected by SARS-CoV-2 via ACE2 and NRP-1-dependent routes cross into fetal tissues more easily, contributing to SARS-CoV-2 vertical transmission.

5. Conclusion

Despite over 3 years of intensive research, our understanding of the mechanisms of SARS-CoV-2 vertical transmission still needs to be advanced. The available data suggest that NRP-1, ACE2, and macrophages are the main points of viral transmission. ACE2 and NRP-1 receptors, highly expressed in placental tissues, facilitate SARS-CoV-2 entry into the host cell and hijack cellular signaling. On the contrary, the expression of TMPRSS2 does not seem to play any key role in SARS-CoV-2 expression. Considering that NRP-1 and ACE2 receptors are present in macrophages and monocytes, SARS-CoV-2-infected macrophages appear to facilitate the viral crossing into placental and fetal tissues. The

expression of both receptors decreases in the later stages of pregnancy, indicating that vertical transmission is most likely during the first and early second trimesters of gestation. Since even asymptomatic infection can manifest as pathological changes in the placenta, pregnant women with confirmed SARS-CoV-2 infection should be mandated for early monitoring and appropriate therapeutic interventions. On the other hand, transmission via breast milk, semen, or upstream vaginal transmission seems to be rare and does not appear to play a significant role in COVID-19 pathogenesis.

Declarations

Funding

This study received no funding.

Institutional review board statement

Due to the nature of this study, the consent of the Bioethical Committee was not required.

Informed consent statement

Due to the nature of this study, an Informed Consent Statement was not required.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of interest

The authors have no conflicts of interest to declare that they are relevant to the content of this article.

References

- Abebe EC, Ayele TM, Muche ZT et al (2021) Neuropilin 1: A novel entry factor for Sars-Cov-2 infection and a potential therapeutic target. *Biologics* 15:143–152. <https://doi.org/10.2147/BTT.S307352>
- Aboudounya MM, Heads RJ (2021) COVID-19 and Toll-like receptor 4 (TLR4): SARS-CoV-2 may bind and activate TLR4 to increase ACE2 expression, facilitating entry and causing hyperinflammation. *Mediators Inflamm* 2021:8874339. <https://doi.org/10.1155/2021/8874339>
- Ahmed S, Zimba O, Gasparyan AY (2020) Thrombosis in coronavirus disease 2019 (COVID-19) through the prism of Virchow's triad. *Clin Rheumatol* 39:2529–2543. <https://doi.org/10.1007/S10067-020-05275-1>
- Alzamora MC, Paredes T, Caceres D et al (2020) Severe COVID-19 during pregnancy and possible vertical transmission. *Am J Perinatol* 37:861–865. <https://doi.org/10.1055/S-0040-1710050>
- Argueta LB, Lacko LA, Yaron Bram Y et al (2022) Inflammatory responses in the placenta upon SARS-CoV-2 infection late in pregnancy. *IScience* 25:104223. <https://doi.org/10.1016/J.ISCI.2022.104223>
- Baergen RN, Burton GJ, Kaplan CG (2022) Benirschke's pathology of the human placenta. Benirschke's pathology of the human placenta. Springer International Publishing. <https://doi.org/10.1007/978-3-030-84725-8>

- Baston-Buest DM, Porn AC, Schanz A et al (2011) Expression of the vascular endothelial growth factor receptor neuropilin-1 at the human embryo-maternal interface. *Eur J Obstet Gynecol Reprod Biol* 154:151–156. <https://doi.org/10.1016/J.EJOGRB.2010.10.018>
- Boncompagni A, De Agostini M, Lugli L et al (2022) Unexpected vertical transmission of SARS-CoV-2: Discordant clinical course and transmission from mother to newborn. *Microorganisms* 10:1718. <https://doi.org/10.3390/MICROORGANISMS10091718>
- Bouachba A, Allias F, Nadaud B et al (2021) Placental lesions and SARS-Cov-2 infection: Diffuse placenta damage associated to poor fetal outcome. *Placenta* 112:97–104. <https://doi.org/10.1016/J.PLACENTA.2021.07.288>
- Boyraz B, James K, Hornick JL et al (2022) Placental pathology from COVID-19—recovered (nonacute) patients. *Hum Pathol* 125:18–22. <https://doi.org/10.1016/J.HUMPATH.2022.04.005>
- Celik O, Saglam A, Baysal B et al (2020) Factors preventing materno-fetal transmission of SARS-CoV-2. *Placenta* 97:1–5. <https://doi.org/10.1016/J.PLACENTA.2020.05.012>
- Centers for Disease Control and Prevention (2023) Care for Breastfeeding People. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/care-for-breastfeeding-people.html>. Accessed 30 June 2023.
- Chambers M, Rees A, Cronin JG et al (2021) Macrophage plasticity in reproduction and environmental influences on their function. *Front Immunol* 11:607328. <https://doi.org/10.3389/FIMMU.2020.607328>
- Chen J, Du L, Wang F et al (2022) Cellular and molecular atlas of the placenta from a COVID-19 pregnant woman infected at midgestation highlights the defective impacts on foetal health. *Cell Prolif* 55:e13204. <https://doi.org/10.1111/CPR.13204>
- Chlamydas S, Papavassiliou AG, Piperi C (2020) Epigenetic mechanisms regulating COVID-19 infection. *Epigenetics* 16:263–270. <https://doi.org/10.1080/15592294.2020.1796896>
- Colson A, Depoix CL, Dessilly G et al (2021) Clinical and in vitro evidence against placenta infection at term by severe acute respiratory syndrome coronavirus 2. *Am J Pathol* 191:1610–1623. <https://doi.org/10.1016/J.AJPATH.2021.05.009>
- Daly JL, Simonetti B, Klein K et al (2020) Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science* 370:861–865. <https://doi.org/10.1126/SCIENCE.ABD3072>
- Donders GGG, Bosmans E, Reumers J et al (2022) Sperm quality and absence of SARS-CoV-2 RNA in semen after COVID-19 infection: A prospective, observational study and validation of the Sperm COVID test. *Fertil Steril* 117:287–296. <https://doi.org/10.1016/J.FERTNSTERT.2021.10.022>
- Edlow AG, Li JZ, Collier A-RY et al (2020) Assessment of maternal and neonatal SARS-CoV-2 viral load, transplacental antibody transfer, and placental pathology in pregnancies during the COVID-19 pandemic. *JAMA Netw Open* 3:e2030455. <https://doi.org/10.1001/JAMANETWORKOPEN.2020.30455>
- European Centre for Disease Prevention and Control (2023) SARS-CoV-2 Variants of Concern as of 29 June 2023. <https://www.ecdc.europa.eu/en/covid-19/variants-concern>. Accessed 30 June 2023.
- Facchetti F, Bugatti M, Drera E et al (2020) SARS-CoV2 vertical transmission with adverse effects on the newborn revealed through integrated immunohistochemical, electron microscopy and molecular analyses of placenta. *EBioMedicine* 59:102951. <https://doi.org/10.1016/J.EBIOM.2020.102951>
- Fallach N, Segal Y, Agassy J et al (2022) Pregnancy outcomes after SARS-CoV-2 infection by trimester: A large, populationbased cohort study. *PLoS One* 17:e0270893. <https://doi.org/10.1371/JOURNAL.PONE.0270893>
- Fenizia C, Saulle I, Di Giminiani M et al (2021) Unlikely SARS-CoV-2 transmission during vaginal delivery. *Reprod Sci* 28:2939–2941. <https://doi.org/10.1007/S43032-021-00681-5>
- Fu Y, Cheng Y, Wu Y (2020) Understanding SARS-CoV-2-mediated inflammatory responses: From mechanisms to potential therapeutic tools. *Virol Sin* 35:266–271. <https://doi.org/10.1007/S12250-020-00207-4>
- Gacci M, Coppi M, Baldi E et al (2021) Semen impairment and occurrence of SARS-CoV-2 virus in semen after recovery from COVID-19. *Hum Reprod* 36:1520–1529. <https://doi.org/10.1093/HUMREP/DEAB026>
- Garcia-Flores V, Romero R, Xu Y et al (2022) Maternal-fetal immune responses in pregnant women infected with SARS-CoV-2. *Nat Commun* 13:320. <https://doi.org/10.1038/S41467-021-27745-Z>
- Gheware A, Ray A, Rana D et al (2022) ACE2 protein expression in lung tissues of severe COVID-19 infection. *Sci Rep* 12:4058. <https://doi.org/10.1038/S41598-022-07918-6>
- Glowacka I, Bertram S, Müller MA et al (2011) Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *J Virol* 85:4122–4134. <https://doi.org/10.1128/JVI.02232-10>
- Gorbalenya AE, Baker SC, Baric RS et al (2020) The species severe acute respiratory syndrome-related coronavirus: Classifying 2019-NCov and naming it SARS-CoV-2. *Nat Microbiol* 5:536–544. <https://doi.org/10.1038/S41564-020-0695-Z>
- Guo HF, Vander Kooi CW (2015) Neuropilin functions as an essential cell surface receptor. *J Biol Chem* 290:29120–29126. <https://doi.org/10.1074/JBC.R115.687327>
- Gychka SG, Brelidze TI, Kuchyn IL et al (2022) Placental vascular remodeling in pregnant women with COVID-19. *PLoS One* 17:e0268591. <https://doi.org/10.1371/JOURNAL.PONE.0268591>
- Hosier H, Farhadian SF, Morotti RA et al (2020) SARS-CoV-2 infection of the placenta. *J Clin Invest* 130:4947–4953. <https://doi.org/10.1172/JCI139569>
- Huang C, Wang Y, Li X et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395:497–506. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5)

- Huang Y, Wang Y, Xu D et al (2022) Characterization of the SARS-CoV-2 coreceptor NRP1 expression profiles in healthy people and cancer patients: Implication for susceptibility to COVID-19 disease and potential therapeutic strategy. *Front Genet* 13:995736. <https://doi.org/10.3389/FGENE.2022.995736>
- Hudak ML, Flannery DD, Barnette K et al (2023) Maternal and newborn hospital outcomes of perinatal SARS-CoV-2 infection: A national registry. *Pediatrics* 151:e2022059595. <https://doi.org/10.1542/PEDS.2022-059595>
- Jackson CB, Farzan M, Chen B et al (2022) Mechanisms of SARS-CoV-2 entry into cells. *Nat Rev Mol Cell Biol* 23:3–20. <https://doi.org/10.1038/S41580-021-00418-X>
- Jafarzadeh A, Chauhan P, Saha B et al (2020) Contribution of monocytes and macrophages to the local tissue inflammation and cytokine storm in COVID-19: Lessons from SARS and MERS, and potential therapeutic interventions. *Life Sci* 257:118102. <https://doi.org/10.1016/J.LFS.2020.118102>
- Jia HP, Dwight C, Look DC, Shi L et al (2005) ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *J Virol* 79:14614–14621. <https://doi.org/10.1128/JVI.79.23.14614-14621.2005>
- Jing Y, Run-Qian L, Hao-Ran et al (2020) Potential influence of COVID-19/ACE2 on the female reproductive system. *Mol Hum Reprod* 26:367–373. <https://doi.org/10.1093/MOLEHR/GAAA030>
- Kapila V, Khalid C (2023) Physiology, placenta. StatPearls Publishing LLC. <https://pubmed.ncbi.nlm.nih.gov/30855916/>
- Karthik K, Senthilkumar TMA, Udhayavel S et al (2020) Role of antibody-dependent enhancement (ADE) in the virulence of SARS-CoV-2 and its mitigation strategies for the development of vaccines and immunotherapies to counter COVID-19. *Hum Vaccin Immunother* 16:3055–3060. <https://doi.org/10.1080/21645515.2020.1796425>
- Karuppan, MKM, Devadoss D, Nair M et al (2021) SARS-CoV-2 infection in the central and peripheral nervous system-associated morbidities and their potential mechanism. *Mol Neurobiol* 58:2465–2480. <https://doi.org/10.1007/S12035-020-02245-1>
- Khalil RA, Granger JP (2002) Vascular mechanisms of increased arterial pressure in preeclampsia: Lessons from animal models. *Am J Physiol Regul Integr Comp Physiol* 283:R29–R45. <https://doi.org/10.1152/AJPREGU.00762.2001>
- Konstantinidou AE, Angelidou S, Havaki S et al (2022) Stillbirth due to SARS-CoV-2 placentitis without evidence of intrauterine transmission to fetus: Association with maternal risk factors. *Ultrasound Obstet Gynecol* 59:813–822. <https://doi.org/10.1002/UOG.24906>
- Kotlyar AM, Grechukhina O, Chen A et al (2021) Vertical transmission of coronavirus disease 2019: A systematic review and meta-analysis. *Am J Obstet Gynecol* 224:35–53.e3. <https://doi.org/10.1016/J.AJOG.2020.07.049>
- Labzin LI, Chew KY, Eschke K et al (2023) Macrophage ACE2 is necessary for SARS-CoV-2 replication and subsequent cytokine responses that restrict continued virion release. *Sci Signal* 16:eabq1366. <https://doi.org/10.1126/SCISIGNAL.ABQ1366>
- Mao Q, Chu S, Shapiro S et al (2022) Placental SARS-CoV-2 distribution correlates with level of tissue oxygenation in COVID-19-associated necrotizing histiocytic intervillitis/perivillous fibrin deposition. *Placenta* 117:187–193. <https://doi.org/10.1016/J.PLACENTA.2021.12.002>
- Mayi BS, Leibowitz JA, Arden T, Woods AT et al (2021) The role of neuropilin-1 in COVID-19. *PLoS Pathog* 17:e1009153. <https://doi.org/10.1371/JOURNAL.PPAT.1009153>
- Megli CJ, Coyne CB (2022) Infections at the maternal-fetal interface: An overview of pathogenesis and defence. *Nat Rev Microbiol* 20:67–82. <https://doi.org/10.1038/S41579-021-00610-Y>
- Menter T, Mertz KD, Jiang S et al (2021) Placental pathology findings during and after SARS-CoV-2 infection: Features of villitis and malperfusion. *Pathobiology* 88:69–77. <https://doi.org/10.1159/000511324>
- Mezouar S, Benammar I, Boumaza A et al (2019) Full-term human placental macrophages eliminate coxiella burnetii through an IFN- γ autocrine loop. *Front Microbiol* 10:2434. <https://doi.org/10.3389/FMICB.2019.02434>
- Mezouar S, Katsogiannou M, Amara AB et al (2021) Placental macrophages: Origin, heterogeneity, function and role in pregnancy-associated infections. *Placenta* 103:94–103. <https://doi.org/10.1016/J.PLACENTA.2020.10.017>
- Mirbeyk M, Saghazadeh A, Rezaei N (2021) A systematic review of pregnant women with COVID-19 and their neonates. *Arch Gynecol Obstet* 304:5–38. <https://doi.org/10.1007/S00404-021-06049-Z>
- Naidoo N, Moodley J, Khaliq OP et al (2022) Neuropilin-1 in the pathogenesis of preeclampsia, HIV-1, and SARS-CoV-2 infection: A review. *Virus Res* 319:198880. <https://doi.org/10.1016/J.VIRUSRES.2022.198880>
- Nobrega Cruz NA, Stoll D, Casarini DE et al (2021) Role of ACE2 in pregnancy and potential implications for COVID-19 susceptibility. *Clin Sci* 135:1805–1824. <https://doi.org/10.1042/CS20210284>
- Pathirathna ML, Samarasekara BPP, Dasanayake TS et al (2022) Adverse perinatal outcomes in COVID-19 infected pregnant women: A systematic review and meta-analysis. *Healthcare* 10:203. <https://doi.org/10.3390/HEALTHCARE10020203>
- Percivalle E, Sammartino JC, Cassaniti I et al (2021) Macrophages and monocytes: ‘Trojan Horses’ in COVID-19. *Viruses* 13:2178. <https://doi.org/10.3390/V13112178>
- Pettiorosso E, Giles M, Cole S et al (2020) COVID-19 and pregnancy: A review of clinical characteristics, obstetric outcomes and vertical transmission. *Austr NZ J Obstet Gynaecol* 60:640–659. <https://doi.org/10.1111/AJO.13204>
- Rackaityte E, Halkias J (2020) Mechanisms of fetal T cell tolerance and immune regulation. *Front Immunol* 11:588. <https://doi.org/10.3389/FIMMU.2020.00588>
- Reyes L, Wolfe B, Golos T (2017) Hofbauer cells: Placental macrophages of fetal origin. *Results Probl Cell Differ* 62:45–60. https://doi.org/10.1007/978-3-319-54090-0_3

- Robbins JR, Bakardjiev AI (2012) Pathogens and the placental fortress. *Curr Opin Microbiol* 15:36–43. <https://doi.org/10.1016/J.MIB.2011.11.006>
- Rojas-Rueda D, Morales-Zamora E (2021) Built environment, transport, and COVID-19: A review. *Curr Environ Health Rep* 8:138–145. <https://doi.org/10.1007/S40572-021-00307-7>
- Roy S, Arup K, Bag AK, Singh RK et al (2017) Multifaceted role of neuropilins in the immune system: Potential targets for immunotherapy. *Front Immunol* 8:1228. <https://doi.org/10.3389/FIMMU.2017.01228>
- Ruan D, Ye ZW, Yuan S et al (2022) Human early syncytiotrophoblasts are highly susceptible to SARS-CoV-2 infection. *Cell Rep Med* 3:100849. <https://doi.org/10.1016/J.XCRM.2022.100849>
- Salamanna F, Maglio M, Landini MP et al (2020) Body localization of ACE-2: On the trail of the keyhole of SARS-CoV-2. *Front Med* 7:594495. <https://doi.org/10.3389/FMED.2020.594495>
- Samavati L, Uhal BD (2020) ACE2, much more than just a receptor for SARS-COV-2. *Front Cell Infect Microbiol* 10:317. <https://doi.org/10.3389/FCIMB.2020.00317>
- Schwartz DA, Baldewijns M, Benachi A et al (2021) Hofbauer cells and COVID-19 in pregnancy: molecular pathology analysis of villous macrophages, endothelial cells, and placental findings from 22 placentas infected by SARS-CoV-2 with and without fetal transmission. *Arch Pathol Lab Med* 145:1328–1340. <https://doi.org/10.5858/ARPA.2021-0296-SA>
- Schwartz DA, Morotti D, Beigi B et al (2020) Confirming vertical fetal infection with coronavirus disease 2019: Neonatal and pathology criteria for early onset and transplacental transmission of severe acute respiratory syndrome coronavirus 2 from infected pregnant mothers. *Arch Pathol Lab Med* 144:1451–1456. <https://doi.org/10.5858/ARPA.2020-0442-SA>
- Sefik E, Rihao Qu R, Junqueira C et al (2022) Inflammasome activation in infected macrophages drives COVID-19 pathology. *Nature* 606:585–593. <https://doi.org/10.1038/S41586-022-04802-1>
- Senapati S, Banerjee P, Bhagavatula S et al (2021) Contributions of human ACE2 and TMPRSS2 in determining host-pathogen interaction of COVID-19. *J Genet* 100:12. <https://doi.org/10.1007/S12041-021-01262-W>
- Seyed Hosseini E, Kashani NR, Nikzad H et al (2020) The novel coronavirus disease-2019 (COVID-19): Mechanism of action, detection and recent therapeutic strategies. *Virology* 551:1–9. <https://doi.org/10.1016/J.VIROL.2020.08.011>
- Shanes ED, Mithal LB, Otero S et al (2020) Placental pathology in COVID-19. *Am J Clin Pathol* 154:23–32. <https://doi.org/10.1093/AJCP/AQAA089>
- Sharps MC, Hayes DJL, Lee S et al (2020) A structured review of placental morphology and histopathological lesions associated with SARS-CoV-2 infection. *Placenta* 101:13–29. <https://doi.org/10.1016/J.PLACENTA.2020.08.018>
- Slomski A (2022) Maternal death rate increased during early COVID-19 pandemic. *JAMA* 328:415. <https://doi.org/10.1001/JAMA.2022.12729>
- Smithgall MC, Liu-Jarin X, Hamele-Bena D et al (2020) Third-trimester placentas of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive women: histomorphology, including viral immunohistochemistry and in-situ hybridization. *Histopathology* 77:994–999. <https://doi.org/10.1111/HIS.14215>
- Takada K, Shimodai-Yamada S, Suzuki M et al (2022) Restriction of SARS-CoV-2 replication in the human placenta. *Placenta* 127:73–76. <https://doi.org/10.1016/J.PLACENTA.2022.07.010>
- The American College of Obstetricians and Gynecologists (2023) COVID-19. <https://www.acog.org/womens-health/covid-19>. Accessed 30 June 2023.
- Vivanti AJ, Vauloup-Fellous C, Prevot S et al (2020) Transplacental transmission of SARS-CoV-2 infection. *Nat Commun* 11:3572. <https://doi.org/10.1038/S41467-020-17436-6>
- Wang S, Wang J, Yu X et al (2022) Antibody-dependent enhancement (ADE) of SARS-CoV-2 pseudoviral infection requires FcγRIIB and virus-antibody complex with bivalent interaction. *Commun Biol* 5:262. <https://doi.org/10.1038/S42003-022-03207-0>
- Watkins JC, Torous VF, Roberts DJ (2021) Defining severe acute respiratory syndrome coronavirus 2 (sars-cov-2) placentitis: a report of 7 cases with confirmatory in situ hybridization, distinct histomorphologic features, and evidence of complement deposition. *Arch Pathol Lab Med* 145:1341–1349. <https://doi.org/10.5858/ARPA.2021-0246-SA>
- Xu Y, Liang Y, Parunov L et al (2020) Combined thrombogenic effects of vessel injury, pregnancy and procoagulant immune globulin administration in mice. *Thromb J* 18:32. <https://doi.org/10.1186/S12959-020-00245-8>
- Yao Y, Xu XH, Jin L (2019) Macrophage polarization in physiological and pathological pregnancy. *Front Immunol* 10:792. <https://doi.org/10.3389/FIMMU.2019.00792>
- Yu X, Lin Q, Qin X et al (2016) ACE2 antagonizes VEGFa to reduce vascular permeability during acute lung injury. *Cell Physiol Biochem* 38:1055–1062. <https://doi.org/10.1159/000443056>
- Zaim S, Chong JH, Sankaranarayanan V et al (2020) COVID-19 and multiorgan response. *Curr Probl Cardiol* 45:100618. <https://doi.org/10.1016/J.CPCARDIOL.2020.100618>
- Zhou P, Yang XL, Wang XG et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579:270–273. <https://doi.org/10.1038/S41586-020-2012-7>