Lung microbiota dysbiosis and the implications of SARS-CoV-2 infection in pregnancy

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***Abstract*:** There are a great number of beneficial commensal microorganisms constitutively colonizing the mucosal lining of the lungs. Alterations in the microbiota profile have been associated with several respiratory diseases such as pneumonia and allergies. Lung microbiota dysbiosis might play an important role in the pathogenic mechanisms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as well as elicit other opportunistic infections associated with coronavirus disease 2019 (COVID-19). With its increasing prevalence and morbidity, SARS-CoV-2 infection in pregnant mothers is inevitable. Recent evidence shows that angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) act as an entry receptor and viral spike priming protein, respectively, for SARS-CoV-2 infection. These receptor proteins are highly expressed in the maternal-fetal interface, including the placental trophoblast, suggesting the possibility of maternal–fetal transmission. In this review, we discuss the role of lung microbiota dysbiosis in respiratory diseases, with an emphasis on COVID-19 and the possible implications of SARS-CoV-2 infection on pregnancy outcome and neonatal health.

Keywords: COVID-19, microbiome, microbiota dysbiosis, pregnancy outcome, SARS-CoV-2

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Introduction

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a betacoronavirus that is known to infect humans and other mammals such as bats. Its zoonotic nature is well proven.[1] The outbreak of the dreadful coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, was first reported in December 2019 in Wuhan, China.[2] The outbreak currently ravaging the world was declared a pandemic on 11 March 2020 by the World Health Organization (WHO).[3] The pandemic has caused a devastating global health and economic burden,[4] with over 3 million deaths as of 31 May 2021.[5] COVID-19 is a complex multisystemic disease, and the spectrum of manifestations can vary from asymptomatic disease to severe acute respiratory distress syndrome, renal dysfunction, hyperimmune state, and sepsis, eventually leading to death.[2,6–8] The pathogenesis of COVID-19 is not fully understood, and its complexity has evolved to the point where microbiota dysbiosis could be implicated in the pathological process.[9–12]

The human microbiome refers to the entire microbial composition of an organ or system, including the microorganisms, their surrounding environmental conditions, genomes, and host interactions.[13] Microbiota is the community of microorganisms colonizing the human body. The human microbiota plays a role in nutrient metabolism, fat store regulation,[14] modulation of the immune response,[8,15] and maintaining host homeostasis.[16] The interplay between commensal microbiota and host immunity is important in

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maintaining mutualism and homeostasis, and perturbation of this interaction could lead to disease.[16,17]

Microbiota dysbiosis has been associated with several respiratory diseases, such as chronic obstructive pulmonary disease,[18,19] asthma,[20,21] cystic fibrosis,[22,23] tuberculosis,[24] and lung cancer.[25] The nasopharynx is the primary site for pathogen colonization, a mechanism that contributes to the onset of respiratory diseases; any imbalance in the mucosal nasopharyngeal microbiota may play a vital role in susceptibility to viral respiratory infections.[26,27] Studies on microbiota composition in a healthy person and patients with respiratory disease suggest that microbiota may play a crucial role in the genesis and clinical development of the disease,[26] including bronchiolitis in infants,[28] and asthma.[29] Edouard *et al*. reported that the microbiota from a healthy nasopharyngeal swab was composed of aero-anaerobic bacteria and was altered during a viral respiratory infection.[26] COVID-19 patients are said to show significant alteration in the gut microbiota during the period of hospitalization and at an all-time point during intensive care.[9,10] Similarly, Man *et al*. recently showed a strong correlation between viral and bacterial microbiota in the upper respiratory tract and the severity and presence of childhood respiratory infection later in life.[27] Thus, alteration of the microbiota profile during SARS-CoV-2 infection might be associated with disease severity.

Emerging and recent studies have shown that lung microbiota dysbiosis could be associated with pulmonary diseases such as pulmonary fibrosis,[30] and this may impact the outcome of COVID-19 cases.[31–33] The imbalance between gut and lung microbiota might compromise host immune response during episodes of SARS-CoV-2 infection, leading to uncontrolled inflammation.[34] However, it is still unclear whether microbiota dysbiosis contributes to the inflammation or whether this is entirely the effect of COVID-19. The focus of this review is twofold. Firstly, to understand the role of lung microbiota dysbiosis on the pathogenesis of respiratory diseases with emphasis on the maternal–fetal transmission of COVID-19, and secondly, to elucidate the impact of SARS-CoV-2 infection on pregnancy, and maternal and child health.

Lung microbiota in respiratory diseases

Respiratory microbiota are often referred to as the gate-keepers to respiratory health, and their involvement in maintaining lung immunity and homeostasis has been studied widely .[35] During the first few weeks of life, distinct lung microbiota exist.[36] For example, the *Staphylococcus aureus* population in the nasopharyngeal microbiota niche declines gradually, and there is a simultaneous increase in potential beneficial commensals such as *Dolosigranulum pigrum* and *Corynebacterium* species.[37] However, a decline in beneficial bacteria has been associated with the risk of pneumonia in children.[38] Bosch *et al*. reported that children delivered by caesarean section were more likely to have delayed development of nasopharyngeal commensals *Dolosigranulum* and *Corynebacterium* profiles early in life, and this might influence their respiratory health later in life.[37] Abundance of nasopharyngeal *Dolosigranulum* (especially *Dolosigranulum pigrum*) and *Corynebacterium* is an indication of a healthy respiratory microbiome.[39] Dysbiosis in this diverse microbiota profile was associated with several respiratory pathologies (Table 1).[40–51] *Dolosigranulum* is a rare opportunistic pathogen that has been confirmed to cause different types of septicemia and pneumonia, while *Corynebacterium* is abundant in children free of *Streptococcus pneumoniae*.[50] Lack of airway *Corynebacterium* might be associated with post-influenza pneumonia.[50,52] The consequences of lung microbiota dysbiosis contribute to the worst pathological outcomes during respiratory viral infection.[53,54] This is further discussed with emphasis on COVID-19.

Lung microbiota dysbiosis in COVID-19 patients

Few studies have examined the role of lung microbiota in mild and severe COVID-19 patients.[31,55–57] A recent study on the analysis of lung microbiota from 20 deceased patients who had severe COVID-19 revealed that the lung microbiota was enriched with bacteria and fungi genera *Acinetobacter* and *Cutaneotrichosporon*, respectively,[56] and that fatal COVID-19 episodes might be associated with complex microbial superinfection such as invasive pulmonary aspergillosis.[58] In addition, gut *Enterobacteriaceae* were also found to predominate in the lungs of deceased COVID-19 patients.[56] This finding might suggest

Table 1. The association of lung microbiota with respiratory diseases.

Model (species)	Respiratory disease	Major findings	References
Human	COPD	Increased *Firmicutes* Proteobacteria Actinobacteria phyla, and *Streptococcus, Corynebacterium, Haemophilus, Pseudomonas, Rothia, Moraxella*, *Lactobacillus* genus in COPD lung microbiota	Erb-Downward *et al.*[40], Huang *et al.*[41], Pragman *et al.*[42], Pragman *et al.*[43], Sze *et al.*[44]
Human	Asthma	Increased eosinophils correlates positively with abundant Actinobacteria (Streptomyces and Propionicimonas)	Huang *et al.*[45]
Human	Allergic rhinitis	High IgE titer correlate with low nasal microbial biodiversity with relatively high *Staphylococcus aureus* and decreased *Propionibacterium acnes*	Hyun *et al.*[46]
Mice	Allergy	Commensal bacteria-derived signal limit lung allergic inflammation	Hill *et al.*[47]
Human	Rhinitis	*Haemophilus*, *Neisseria*, and *Moraxella* increased significantly in children with rhinitis. *Moraxella* spp. is associated with children with Rhinitis interacting mite sensitization. Airway *Leptotrichia* is found in amount in mite-sensitized asthma in children	Chiu *et al.*[48,49]
Human	Allergic rhinitis	Airway *Haemophilus* spp. are positively correlated with IgE levels	Chiu *et al.*[49]
Human	Influenza	Enriched *Staphylococcus* and *Dolosigranulum* taxa in the nasopharyngeal region	Ding *et al.*[50]
Human	Acute respiratory tract infection (RSV or Rhinovirus)	Abundant airway *Haemophilus* in RSV-positive infants	Rosas-Salazar *et al.*[51]

COPD, Chronic obstructive pulmonary disease.

the possibility of intestinal to respiratory microbiome crosstalk or migration. While the mechanism by which this could occur remains elusive,[59] it is plausible that endotoxin secreted by pathogenic *Enterobacteriaceae* affect gut and lung epithelial cells, thereby leading to heightened pulmonary inflammation.[56] De Maio *et al.* reported that patients with mild COVID-19 showed no statistical difference in their nasopharyngeal bacterial profile compared with non-infected patients.[60] As microbiota dysbiosis occurs in severe COVID-19 patients, it is not surprising that disturbance in lung microbiota might play a role in SARS-CoV-2 pathogenesis.

Pathogenesis of SARS-CoV-2 infection

Research on the pathogenesis of SARS-CoV-2 infection in humans is still evolving. The use of animal models is vital for understanding SARS-CoV-2 pathogenesis.[61] Using transgenic mice expressing human angiotensin-converting enzyme 2 (ACE 2), Jiang *et al.* reported that SARS-CoV-2 viral particles were found in the lung and brain region.[61] They also found that, between the fourth and seventh day of infection, there was significant body weight loss, host immune and cardiac dysfunction, respiratory distress, and even death.[61] Likewise, Israelow *et al.* reported heightened inflammatory interferon signatures in the lungs similar to COVID-19 patients.[62] Mechanistically, SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2), and it also utilizes transmembrane protease serine 2 (TMPRSS), both expressed on peripheral tissues, including lungs, to gain entry into the host,[63–65] thereby affecting host physiological functions (Figure 1). Following this, SARS-CoV-2 can co-infect multiple organs apart from the lungs. The full understanding of human SARS-CoV-2 pathogenesis is currently incomplete and is a rapidly developing area of science.[66,67]

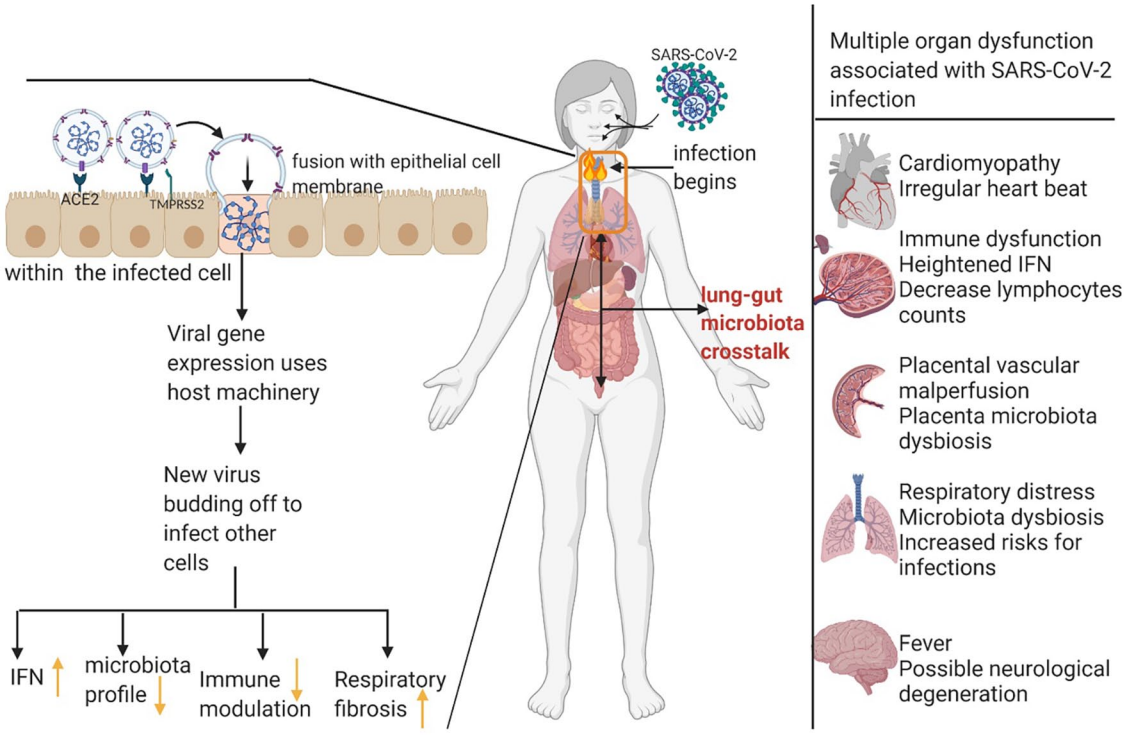

Figure 1. Representation of possible pathogenesis of SARS-CoV-2 and effects on mammalian organs. SARS-CoV-2 binds to ACE2, and protease activity of TMPRSS2 facilitates entry into the lung epithelial cells. The pathogenesis of SARS-CoV-2 induces heightened inflammation, microbiota dysbiosis, and possible adverse outcomes such as respiratory distress, and cardiomyopathy, thus complicating host health. (Figure created in Biorender).
ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2.

SARS-CoV-2 receptors and target cells

ACE2 is the main entry receptor for SARS-CoV-2. The virus utilizes TMPRSS2 as viral spike (S) protein priming.[64] These receptor proteins are expressed predominantly in pulmonary and extrapulmonary organs, including lung type II pneumocytes, nasal goblet cells, placenta,[65] ileal absorptive enterocytes,[68] intestine,[69] liver, and heart.[63] They are also expressed widely in specific cell types, such as decidual stromal cells, placental cytotrophoblasts, and syncytiotrophoblasts at the maternal-fetal interface.[70,71] ACE2 is a carboxypeptidase that cleaves angiotensinogen into smaller angiotensin peptides. Early pathogenesis of COVID-19 requires the attachment of the viral S protein to epithelial ACE2, thereby inducing endocytosis, which is followed by priming the viral S protein by TMPRSS2 protease activity, facilitating SARS-CoV-2 entry into the host cell.[63] Apart from ACE2 and TMPRSS2, only a few other proteins, including transducin-like enhancer protein 3 (TLE3) and lysyl oxidase (LOX), which interacts with SARS-CoV-2, were found in an *in silico* analysis to be upregulated in early and term placental tissue, respectively.[65] LOX is expressed highly in both human fetal membranes and mesenchymal cells of the placenta.[65,72] LOX family proteins are extracellular enzymes that participate in reproduction, and an altered expression of LOX protein is associated with endometriosis,[73] impaired placental trophoblast migration, and preeclampsia.[74] SARS-CoV-2 interacts with proteins that are involved in placental function, implantation, and successful decidualization, thus implying a probable route of fetal infection.

Possible maternal–fetal transmission route

The transmission of SARS-CoV-2 from a pregnant mother to a developing fetus is quite rare but possible.[70] With increasing research on the COVID-19 pandemic, more findings suggest that

vertical transmission of SARS-CoV-2 is possible.[75] For instance, ACE2 and TMPRSS2 are expressed at the maternal–fetal interface, which indicates the possibility of *in utero* transmission.[76,77] Similarly, Fenizia *et al*. reported that SARS-CoV-2 genome was found in the umbilical cord blood, at-term placentas, breast milk, and vaginal mucosa in 1 out of 31 pregnant mothers involved in their study.[70] Babies born to mothers who tested positive for COVID-19 have a detectable amount of the virus-specific antibodies in their sera.[78] Vivanti *et al*. recently reported possible transmission of SARS-CoV-2 between pregnant mothers and their developing fetuses using comprehensive immunological and virological techniques.[79] All samples collected, including amniotic fluid, were positive for SARS-CoV-2, and the developing fetuses showed irregular fetal heartbeats,[79] accompanied by neurological defects such as encephalitic symptoms early in life.[80] In a longitudinal study, mothers who tested positive for COVID-19 showed a positive serological test for IgM in breast milk between 3 and 68 days after the onset of COVID-19 symptoms.[81] Consecutively, others reported IgM at day 8 and IgG on day 28 in the breast milk of nursing infected mothers, cord blood, and neonatal serum.[82] However, there was no detectable trace of SARS-CoV-2 found in breast milk.[82] At the moment, the transmission of SARS-CoV-2 *via* breast milk remains inconclusive.[70,78] It was suggested that proper hygiene during breastfeeding might contribute to a low risk of transmitting SARS-CoV-2 in neonates.[83]

Microbiota dysbiosis during pregnancy and birth

Pregnancy is a physiological state that involves changes in hormonal homeostasis, immunity, metabolic processes, and microbiota composition in order to support fetal growth and development.[84] Until recently, there was speculation that developing fetuses are germ-free since the womb and placenta are sterile.[84] However, the uterus is, after all, not sterile but home to most beneficial commensals, suggesting that fetuses are exposed to commensals during development, and this, in turn, might influence fetal immune development.[85] For the first time, Al Alam *et al*. reported possible traces of human fetal and placenta microbiome as early as the first trimester in pregnancy, and these microbiotas inhabit the fetal lung.[86] The placenta has also been shown to consist of a diverse microbiota profile.[87] Using genomic DNA sequencing, Parnell *et al*. reported that distinct microbiota diversities exist in the placenta.[87] Their findings also showed that *Ralstonia insidiosa* and *Mesorhizobium* spp. were abundant in both the placental villi and basal plate while the *Lactobacillus* spp. were abundant in the fetal amniotic membrane. The composition of microbiota early and later in life of these neonates might depend on the mode of birth (either *via* vaginal delivery or caesarean section) and maternal nutrition.[88–90] We postulate that distinct fetal lung microbiota might be established in the early phases of pregnancy.

The importance of microbiota in health has been well established.[91] Lactobacilli and Prevotella dominate the vaginal microbiota,[92,93] and Proteobacteria and Actinobacteria are abundant in gut microbiota. In contrast, Actinobacteria, Fermicutes, and Bacteroides dominate oral microbiota.[94] Disturbance in the oral, intestinal, placenta, and vaginal microbiota during pregnancy either as a result of infection or stress such as hormonal imbalance might lead to complications for the baby, including metabolic programming during development,[95] preterm birth,[96] and altered neurological development.[94,97]

Potential implications of COVID-19 in pregnancy

It is possible that COVID-19 might have an adverse impact on pregnant mothers and their babies, but the exact mechanism is still unknown.[98,99] SARS-CoV-2 infection during pregnancy might affect placental morphology, thereby impacting the pregnancy adversely (Figure 2). The placenta is a unified organ responsible for mother to fetus nutrient transport, and it accommodates numerous commensal microorganisms. Placental microbiota dysbiosis during pregnancy might lead to undesired outcomes.[100–102] There is increasing evidence that SARS-CoV-2 is detected in placental tissue and amniotic fluid in preterm fetuses born to SARS-CoV-2 infected mothers.[103,104] Histological analysis of placental morphology in neonates born to SARS-CoV-2 infected mothers revealed fetal vascular malperfusion,[105] maternal vascular malperfusion,[106] heightened macrophage, and lymphocytes infiltration.[103] Heightened placental

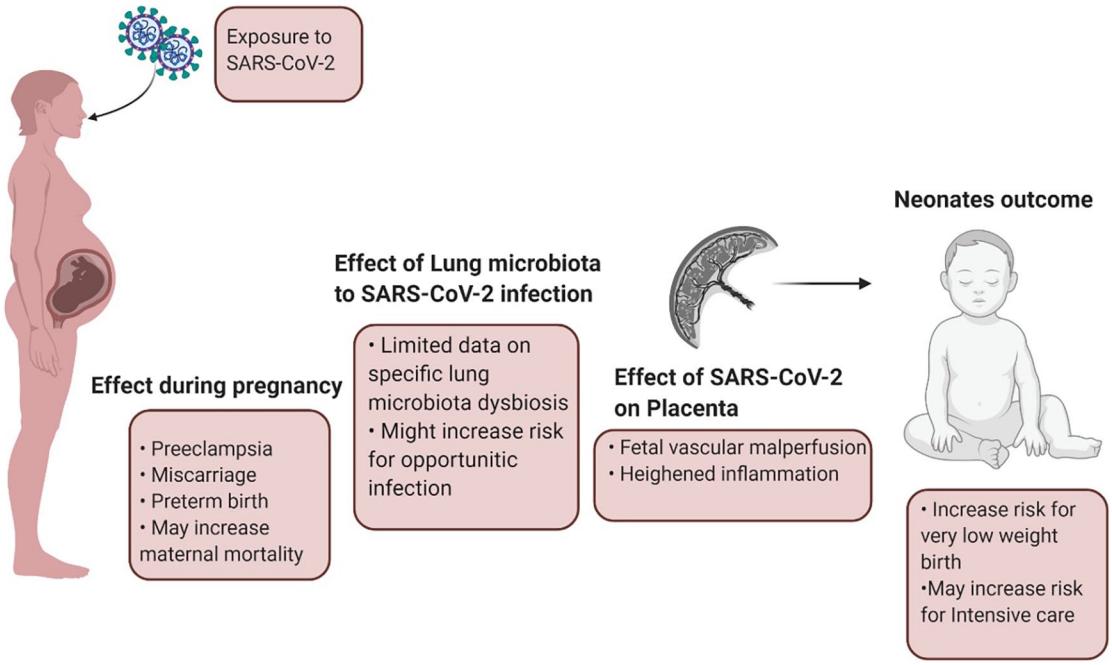

Figure 2. Representation of exposure to SARS-CoV-2 infection during pregnancy and neonate/fetus outcome. SARS-CoV-2 infection in pregnancy may induce vascular malperfusion in the placenta. This might alters fetal growth and development, which may cause negative pregnancy outcome, as well as have adverse effects in neonates health later in life. (Figure created in Biorender).
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

inflammation might result in early-onset of preeclampsia, poor maternal condition, and adverse birth outcomes.[103]

There are reports that describe the outcomes of neonates born to mothers with SARS-CoV-2.[107] COVID-19 affects successful fetal development and might cause preeclampsia, miscarriages, fetal growth restriction, and preterm birth.[107,108] More pregnant women with COVID-19 are now opting for caesarean section during the third trimester because of the uncertainty of mother-child transmission *via* vaginal delivery.[107,109] However, babies delivered by a caesarean section might lose out on inheriting most of the beneficial vaginal microbiome. Pregnant women are in an immunosuppressed state and recent studies show that they are susceptible to respiratory diseases such as severe pneumonia.[109] When infected with SARS-CoV-2, they present with clinical symptoms such as fever, cough,[83] myalgia, and rashes[75] and laboratory findings show elevated C-reactive protein and lymphocytopenia.[110]

Emerging research has also shown that SARS-CoV-2 infection during pregnancy might cause miscarriages,[111] small for gestational age, low birthweight (<2500 g), preterm births,[70] and even fetal death.[112,113] Of the 125 pregnant women admitted to an intensive care unit in a multicenter unit in Turkey, about 86.5% of neonates born to such mothers were isolated in the neonatal intensive care unit for respiratory support.[114] Symptoms associated with neonates born to SARS-CoV-2 infected mothers include respiratory distress, gastrointestinal disturbance, fever, irregular heart rate, abnormal liver function, poor immune function, multiple organ failure,[115] unexplained rashes, and facial ulceration.[116] Data from a large cohort (n = 91,412) of women of reproductive age in the United States (US) with laboratory-confirmed COVID-19 revealed that the incidence of the disease might be higher in pregnant women (31.5%) than non-pregnant women (5.8 %),[117] and there was a possibility that these figures might continue to rise. In another smaller cohort study involving 46 patients, it was found that COVID-19 patients with underlying medical conditions such as obesity had an increased risk for pregnancy complications.[118] With the emerging findings on possible maternal–fetal transmission and pregnancy outcome, more research is warranted

on the effects of SARS-CoV-2 infection during pregnancy and neonatal health.

Conclusion and future direction

Lung microbiota dysbiosis is associated with respiratory distress and increases the risk of respiratory infection. SARS-CoV-2 infection during the gestational period affects maternal health and may cause severe complications for the developing fetus, such as metabolic programming and restricted growth leading to preterm birth. With the emerging findings on possible maternal–fetal transmission and adverse pregnancy outcomes in patients infected with SARS-CoV-2, we postulate that lung-gut microbiota crosstalk exists and maternal–fetal microbiota exchange during pregnancy and birth may be a signature for neonatal, infant, and child health. However, it is premature to speculate on the effect of SARS-CoV-2-driven lung microbiota dysbiosis and its eventual impact on pregnancy outcomes due to maternal infection. Future larger scale, longitudinal, population studies should further explore: (1) the mechanisms whereby SARS-CoV-2 influences specific maternal lung microbiota during pregnancy, (2) whether such specific dysbiosis is transferred during pregnancy to the developing fetus, and (3) whether there is a correlation between specific fetal microbiota profiles at different trimesters with pregnancy outcome and neonatal health. Results of these emerging studies might be useful in providing guidelines for managing SARS-CoV-2 infection during pregnancy.

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