The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can infect the human placenta. In this study, the authors from Italy performed immunohistochemical analyses to determine whether SARS-CoV-2 alters markers involved in vascular damage and the autophagic process in the placental tissue of unvaccinated women infected with SARS-CoV-2.

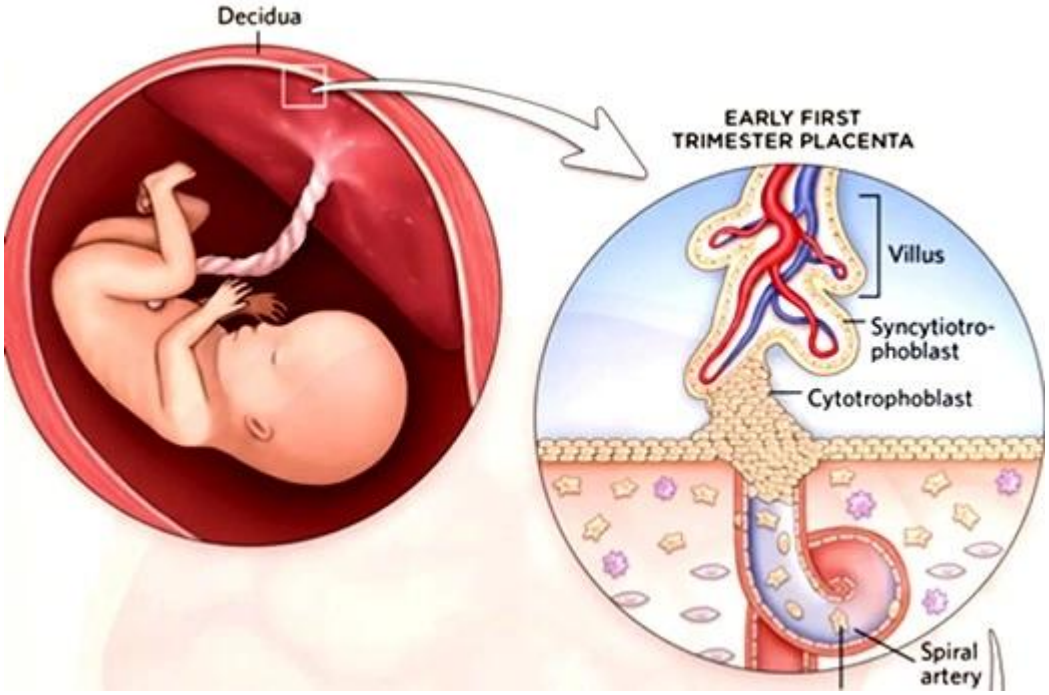
SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) protein. Two host-cell factors are important for SARS-CoV-2 viral entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which is bound by the S-protein, and transmembrane serine protease 2 (TMPRSS2), which cleaves the S protein, allowing this binding to take place. The ACE2 receptor is expressed in the placenta.

In addition to ACE2 and TMPRSS2, it has been reported that the S protein engages other cell-surface factors proposed to serve as attachment factors promoting SARS-CoV-2 entry. The cluster of differentiation 147 (CD147) is a transmembrane protein reported to be involved in virus entry and considered in some studies as a putative alternative receptor. In the placenta, CD147 is involved in the implantation, invasion, and differentiation of human trophoblasts. It is also involved in other viral infections, such as the HIV-1 infection.

The normal vascular pattern of the placenta includes vasculogenesis and angiogenesis, which are associated with the physiological expression of vascular endothelial growth factor (VEGF). It has been reported that SARS-CoV-2 infection significantly alters the placental vasculature, resulting in diminished maternal vascular perfusion and insufficient blood flow to the fetus. The placental SARS-CoV-2 infection also alters coagulation and elevates thrombin production. In 2022, the study by Gychka SG et al. demonstrated severe vascular remodeling of placental arteries, including severe thickening of the vessel walls and the occlusion of the vessel lumen in women infected with SARS-CoV-2 during pregnancy. https://discovermednews.com/severe-vascular-remodeling-of-placental-arteries-in-women-with-sars-cov-2-during-pregnancy/

The autophagic process plays an important role in the early embryonic stages and contributes to the balance of the maternal-fetal components during normal placental development. LC3, or microtubule-associated protein 1 Light Chain 3, is expressed in the autophagosome membrane during the autophagic process and could elicit rapid degradation of mRNAs. SARS-CoV-2, but not SARS-CoV, has been shown to induce autophagy and the accumulation of autophagosomes, which appear to be central to viral replication and virion release. The authors emphasized the possible dual role of autophagy in the SARS-CoV-2

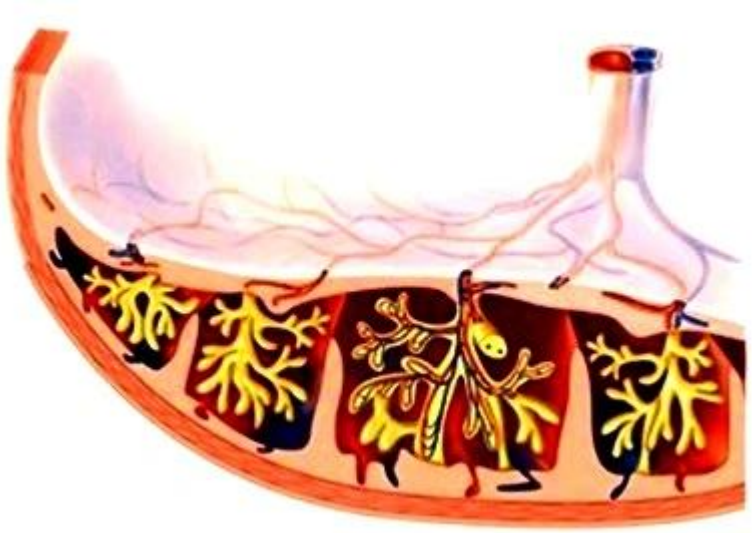
infection. It could act as a defense mechanism, degrading viral components to limit viral replication, but, the virus may drive the autophagic process to promote its survival and replication. The LC3B variant is involved in the autophagic response to SARS-CoV-2 infection.



About the study

The authors performed an immunohistochemical analysis of placental samples obtained from three groups: women who were positive for SARS-CoV-2 at delivery, women who had COVID-19 during pregnancy but who were negative for SARS-CoV-2 at delivery and control women who had given birth before 2019. SARS-CoV-2 infection was confirmed by reverse transcription polymerase chain reaction (rt-PCR). After delivery, placental samples were immediately collected, fixed, and then analyzed using standard immunohistochemistry to investigate SARS-CoV-2 S protein, ACE2 receptor, CD147, endothelial CD34 marker, VEGF, and LC3B. The presence of the virus was assessed by the expression of viral S protein,

vascular impairment by expression of VEGF and CD34, and the presence of autophagy by expression of LC3B. Multiplexed immunohistochemical consecutive staining on a single slide (MICSSS) was used to examine the co-expression of antigens in the same specimen.



Results

The study included 15 placental samples from 5 women with positive rt-PCR for SARS-CoV-2 at delivery, 5 women who had COVID-19 during pregnancy but who were negative for SARS-CoV-2 at delivery, and 5 control women who gave birth before 2019. A period of negativity ranged from 199 to 41 days before delivery with a mean of 88.4 ± 71.0 days. None of the pregnant women were vaccinated against SARS-CoV-2.

The participants did not differ regarding age, body mass index, or gestational age at delivery. One woman from the SARS-CoV-2 PCR- group and another from the control group, had type 2 diabetes. Besides that, the enrolled women had no comorbidities, preexisting diseases such as hypertension or heart problems, or pregnancy-related diseases such as preeclampsia. Regarding COVID-19 complications, one woman reported fever and respiratory symptoms two months before delivery, while another with diabetes developed fever and dyspnea that required the use of oxygen therapy.

Biochemical data showed no significant differences in D-Dimer and aPTT values between the groups and these parameters were within normal ranges. Two groups with previous or

ongoing SARS-CoV-2 infection showed higher fibrinogen levels than the control group.

All samples from the SARS-CoV-2+ group contained the S protein in decidua and chorionic villi, but, it was absent in the placental samples from SARS-CoV-2 negative convalescents and controls. The S protein was well outlined between the villi, particularly at the syncytiotrophoblast level. The islets of the S protein in the decidua were mainly located toward its external surface.

The ACE2 receptor was expressed in the chorionic villi, the surface of the syncytiotrophoblast, and the decidua. The ACE2 expression was slightly higher in the placentas of SARS-CoV-2 positive women than in controls and SARS-CoV-2 negative convalescents. The expression of transmembrane protein CD147 was increased in SARS-CoV-2 positive samples, while its expression was lower in SARS-CoV-2 negative placentas and controls. The S protein expression correlated positively with the ACE2 expression in the placental tissue, but its correlation with the CD147 was less significant.

CD34 characterized the areas of the vascular endothelium in all three groups. It was expressed in the endothelium, near the syncytiotrophoblast, and on the surface of the vascular endothelium of the decidua. Its expression was greater in villi than in decidua.

VEGF expression was higher in the placentas of SARS-CoV-2 positive women, both in the villi and decidua than in SARS-CoV-2-negative placentas and controls. In the villi, VEGF was expressed at the level of syncytiotrophoblast and vascular endothelium, whereas in the decidua VEGF was mainly detectable in the vascular endothelium. The expression of the S protein correlated positively with CD34 or VEGF in both villi and decidua.

The expression of LC3B protein in the villi and decidua was increased in SARS-CoV-2-positive samples compared to SARS-CoV-2-negative placentas and controls. A positive correlation between the S protein and LC3B expression confirmed the activation of autophagy during infection. According to the authors, S protein and LC3B in the endothelial cells of the villi and decidua of SARS-CoV-2-positive placental samples indicate an extensive infection.

Conclusion

The IHC analysis confirmed the presence of the SARS-CoV-2 S protein only in SARS-CoV-2 PCR+ samples. The S protein was concentrated in the syncytiotrophoblast and the decidua. According to the authors, the increased expression of VEGF and the endothelial cell marker

CD34 indicates alterations, disarrangements, or remodeling of normal vasculature, associated with vascular endothelial injury and inflammation, presumably endothelitis. The MICSSS method confirmed the colocalization of the S protein with VEGF and CD34. The expression of the autophagosomal marker LC3B was increased in SARS-CoV-2-positive placentas at the trophoblast level, but not in placentas of SARS-CoV-2-negative convalescents.

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Journal Reference

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