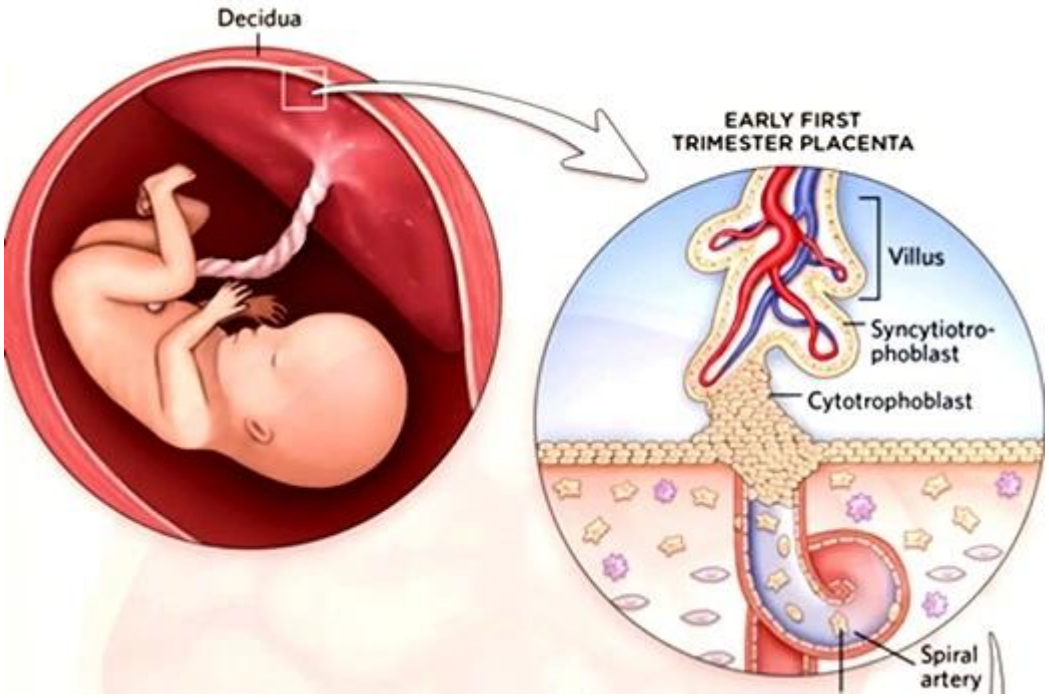
The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can infect the human placenta. In this study, Italian authors performed immunohistochemical analyses to determine whether SARS-CoV-2 can alter markers involved in vascular damage and the autophagic process in the placental tissue of unvaccinated 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) proteins. Two host-cell factors are important for SARS-CoV-2 viral entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which the S-protein binds, 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.

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. This results 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, 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 between the maternal and fetal components during normal placental development. 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. Light Chain 3 (LC3), a microtubule-associated protein, is expressed in the membrane of the autophagosome during the autophagic process and could elicit rapid degradation of mRNAs. The variant LC3B is involved in the autophagic response to SARS-CoV-2 infection.

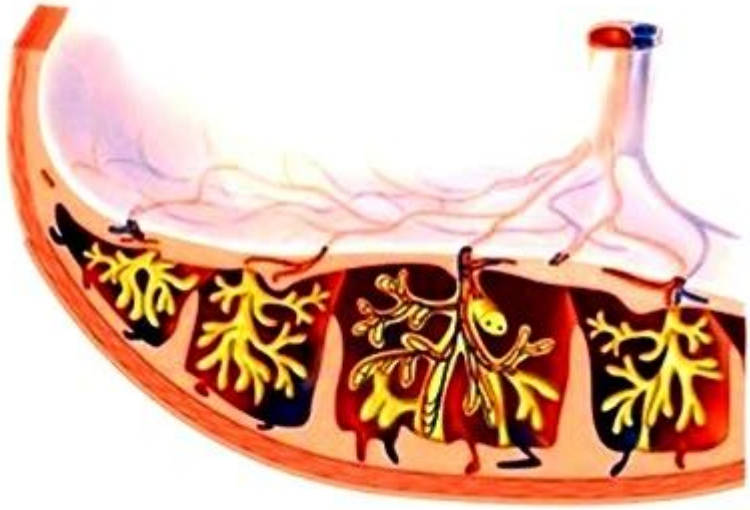
The authors emphasized the possible dual role of autophagy in the SARS-CoV-2 infection. It could be a defense mechanism, degrading viral components to limit viral replication. However, the virus could also drive the autophagic process to promote its survival and replication.



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 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, vascular endothelial growth factor (VEGF), and LC3B.

The SARS-CoV-2 presence was assessed by the S protein expression. Vascular impairment was assessed by VEGF and CD34 expression, and the presence of autophagy was assessed by expression of LC3B. The co-expression of antigens in the same specimen was evaluated by multiplexed immunohistochemical consecutive staining on a single slide (MICSSS).



Results

The study included 15 placental samples from 15 women: 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 was vaccinated against SARS-CoV-2.

The participants did not differ regarding age, body mass index, or gestational age at delivery. One woman with positive rt-PCR for SARS-CoV-2 and another from the control group had type 2 diabetes. Besides that, the enrolled women had no comorbidities, pre-existing 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.

Laboratory tests demonstrated no significant differences in D-dimer and aPTT values between the groups; these parameters were within normal ranges. Two groups with previous or ongoing SARS-CoV-2 infection showed higher fibrinogen levels than the control group.

Immunohistochemistry Results

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.

The S protein was detected in the decidua and chorionic villi of all placenta samples from women positive for SARS-CoV-2. 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.

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. CD34 expression was greater in villi than in decidua.

Placenta samples from women positive for SARS-CoV-2 had higher VEGF expression, both in the villi and decidua, than placentas of SARS-CoV-2-negative women 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 expression in both, villi and decidua.

Placenta samples from women positive for SARS-CoV-2 had higher LC3B protein expression in the villi and decidua, 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.

Conclusion

This study confirmed the presence of the SARS-CoV-2 S protein only in placenta samples from women positive for SARS-CoV-2. The S protein was concentrated in the syncytiotrophoblast and the decidua. Placenta samples from women positive for SARS-CoV-2 also had higher VEGF and LC3B protein expression in the villi and decidua. These results confirmed the co-localization of the S protein with VEGF and CD34 in placentas of women

positive for SARS-CoV-2.

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.

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Journal Reference

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