



Maternal-fetal transmission of SARS-CoV-2, viral tropism for various fetal brain cells, and a concerning degree of brain gliosis were observed in the brains of in utero-infected mouse fetuses | 1

Numerous viral pathogens, including the Zika virus, herpes simplex virus, the human immunodeficiency virus, and others, possess the capability to cross the placenta and infect fetal tissues, resulting in mild to severe neurological complications. Recent findings have shown that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can also infect the placenta and fetal tissue. In this study, the authors from the United States used a mouse model to investigate the maternal-fetal transmission of the delta variant of SARS-CoV-2 and the impact of SARS-CoV-2 on the developing fetal brain.

Previous studies reported numerous complications in mothers infected with SARS-CoV-2 and their newborns. The complications observed in mothers include preeclampsia, preterm birth, and miscarriages, whereas complications observed in newborns exposed to COVID-19 *in utero* include neurodevelopmental delay, motor deficits, seizures, and microcephaly. A recent study described 2 cases of second-trimester maternal SARS-CoV-2 infection with placentitis that triggered an inflammatory response and oxidative stress injury to the fetoplacental unit. This affected the fetal brain and resulted in progressive neurodevelopmental sequelae in their neonates. The S1 subunit of the SARS-CoV-2 spike (S) protein and nucleocapsid (N) protein were detected in both placentas and throughout the brain of the deceased infant.

<https://discovermednews.com/neurodevelopmental-sequelae-microcephaly-in-newborns-after-in-utero-exposure-to-sars-cov-2/>

Of note, studies have also shown severe vascular remodeling of placental arteries, including severe thickening of the vessel wall and the occlusion of the vessel lumen in women infected with SARS-CoV-2 during pregnancy, as well as co-localization of SARS-CoV-2 spike protein with increased expression of vascular and autophagy markers in the placental tissue of unvaccinated women infected with SARS-CoV-2.

<https://discovermednews.com/severe-vascular-remodeling-of-placental-arteries-in-women-with-sars-cov-2-during-pregnancy/>

<https://discovermednews.com/colocalization-of-sars-cov-2-spike-protein-with-markers-of-vascular-damage-and-autophagy-in-placentas/>

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About the Study and Results

To investigate the vertical transmission of SARS-CoV-2 from mother to fetus and the offspring pathology, the authors used heterozygous female hACE2-KI mice to generate litters containing both hACE2+ (SARS-CoV-2-susceptible) and hACE2- (SARS-CoV-2-resistant) fetal genotypes. The 12-week-old nonpregnant heterozygous hACE2-KI female mice were intranasally infected with mock or 10^3 or 10^5 focus-forming units (FFU) of delta variant SARS-CoV-2. After viral infection, mice were monitored for four days for morbidity (body weight loss) and mortality (survival). On the fourth day after the infection, the animals were euthanized, and their lungs, spleen, and brain were harvested for analysis of viral load by reverse transcription polymerase chain reaction (RT-qPCR) test.

The heterozygous female hACE2-KI mice were crossed to either hemizygous hACE2-KI males or WT males to generate desired fetal genotypes, hACE2+ and hACE2- fetuses. The first time point was the mid-gestational stage of E14.5, before the astroglialogenesis. This time point approximately corresponds to the end of the first trimester and the beginning of the second trimester in humans. The second time point of E18.5 is the late gestational stage after astroglialogenesis. This time point approximately corresponds to the beginning of the third trimester in humans. Both time points occur after placental formation. Body weights of pregnant mice, increased fur ruffling, and hunched posture were used as clinical signs of the SARS-CoV-2 infection.

Pre-term birth was observed in approximately 45% of infected litters.



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Mice with the mock infection and those infected with 10^3 or 10^5 FFU of the delta variant SARS-CoV-2 did not display any weight loss or signs of disease. However, mice infected with 10^5 FFU of SARS-CoV-2 lost 20% of their pre-infection body weight within 48 hours after infection and exhibited signs of disease. However, their body weight recovered to 100% 72 hours after the infection.

In mothers infected with 10^3 or 10^5 FFU, RT-PCR showed lung and spleen infection 4 days after SARS-CoV-2 infection. Viral transcription (an indicator of viral replication) was detected in the lungs of mothers infected with 10^5 FFU. Interestingly, SARS-CoV-2 infection without replication was detected in the brains of pregnant mice at both time points.

At the E14.5 time point, 22% of hACE2+ fetuses had a positive viral load in abdominal, thoracic, and heart tissues, and only 5% had a positive viral load in their brains.

At the E18.5 (later time point), 60% of hACE2+ fetuses had a positive viral load in abdominal, thoracic, and heart tissues, and 58% had a positive viral load in their brains. These findings show that maternal-fetal transmission of SARS-CoV-2 occurred mostly in the later stages of pregnancy and that hACE2 expression was necessary for SARS-CoV-2 infection in this model.

The virus was not detected in any tissue of the hACE2- fetuses.

The viral load was significantly higher in male fetuses than in female fetuses.

12-27% of brain cells in hACE2+ fetuses were positive for the SARS-CoV-2 S protein. The brains of hACE2- fetuses and the mock-infected group were negative for the S protein. This shows that hACE2 facilitates SARS-CoV-2 infection in this model system.

Cells positive for the SARS-CoV-2 S protein in the fetal brains were positive for different cell markers. About 19% were positive for the immature neuron marker, 13% for the mature neuron marker, 31% for the choroid plexus cell marker, 28% for the astrocyte marker, and 27% for the microglia marker. The S protein was found within brain blood vessels in cortical and hippocampal regions of fetal brains. Co-localization of the S protein and CD31, the marker for endothelial cells, confirmed the blood vessel infection. According to the authors, these results suggest that SARS-CoV-2 entered the fetal brain *via* the circulatory system, indicating the same transmission route in humans.

The rate of brain cell death in fetuses infected with SARS-CoV-2 did not differ from the mock-infected group. But, seven days after SARS-CoV-2 infection, cortical and hippocampal



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gliosis significantly increased in the brains of SARS-CoV-2-infected pups. The astrocytes expressed glial fibrillary acidic protein (GFAP), typical of astrogliosis, while microglia displayed a “bushy” morphology with larger cell bodies, typical of a reactive state. At that time, the S protein was not detected, indicating that the SARS-CoV-2 infection had cleared.

Conclusion

This study has shown maternal-fetal transmission of SARS-CoV-2 in later stages of mouse pregnancy, at time points corresponding to the second and third trimesters of human pregnancy. The level of infection was much higher at a time point that corresponds to the third trimester in humans. SARS-CoV-2 demonstrated tropism for various fetal brain cells, like endothelial cells of blood vessels, barrier cells of the choroid plexus, neurons, and glial cells. A concerning degree of gliosis was observed in the brains of infected fetuses, but there was no increase in cell death. Notably, male fetuses showed higher rates and levels of viral infection compared to female fetuses. In this model, hACE2 expression was necessary for SARS-CoV-2 infection.

The authors concluded that the observed deleterious effects of maternal-fetal transmission on fetal neurodevelopment may be more severe in humans, who are more susceptible to SARS-CoV-2 infection than mice. These findings, therefore, have important implications for pregnancy and fetal complications in humans following prenatal exposure to SARS-CoV-2.

This article was published in Brain Behavior Immunology.

Journal Reference

McMahon CL, Castro J, Silvas J, et al. Fetal brain vulnerability to SARS-CoV-2 infection. *Brain Behav Immun.* 2023 Aug; 112: 188–205. (Open Access)
<https://doi.org/10.1016/j.bbi.2023.06.015>

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