Diminished Antiviral Innate Immune Gene Expression in the Placenta Following a Maternal SARS-CoV-2 Infection

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Title: Diminished Antiviral Innate Immune Gene Expression in the Placenta Following a Maternal SARS-CoV-2 Infection

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Condensation: COVID-19 impairs placental immunity, which remains suppressed for weeks to months following a maternal SARS-CoV-2 infection.

Short Title: Placental innate immune suppression after maternal COVID-19

AJOG at a Glance

Why was this study conducted? To evaluate the impact of a maternal SARS-CoV-2 infection on the placental innate immune response.

Key Findings:

1) We demonstrate a significant reduction in the gene expression of numerous innate immune mediators critical for antiviral host defense in the placental tissues from a large cohort of pregnant women diagnosed with COVID-19 during their pregnancy.

2) Placental innate immune defenses were negatively impacted regardless of COVID-19 disease severity, gestational age at COVID-19 diagnosis and the time interval between diagnosis and delivery.

What does this add to what is known? Even after having mild COVID-19 disease, the placenta may have a significantly impaired immune response, which may increase the risk for other infections in pregnancy or reflect a broader decline in placental function.

Keywords: SARS-CoV-2, COVID-19, placenta, fetus, pregnancy, immune response, chorionic villous, chorioamniotic membrane
Abstract

Background: Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is associated with critical illness requiring hospitalization, maternal mortality, stillbirth, and preterm birth. SARS-CoV-2 has been shown to induce placental pathology, however, substantial gaps exist in our understanding of the pathophysiology of COVID-19 disease in pregnancy and the long-term impact of SARS-CoV-2 on the placenta and fetus. To what extent a SARS-CoV-2 infection of the placenta alters the placental antiviral innate immune response is not well understood. A dysregulated innate immune response in the setting of maternal COVID-19 disease may increase risk for inflammatory tissue injury or placental compromise and contribute to deleterious pregnancy outcomes.

Objective: We sought to determine the impact of a maternal SARS-CoV-2 infection on placental immune response by evaluating gene expression of a panel of six antiviral innate immune mediators that act as biomarkers of the antiviral and interferon cytokine response. Our hypothesis was that a SARS-CoV-2 infection during pregnancy would result in an upregulated placental antiviral innate immune response.

Study Design: We performed a case control study on placental tissues [chorionic villous (CV) tissues and chorioamniotic membrane (CAM)] collected from pregnant patients with (N=140) and without (N=24) COVID-19 disease. We performed real-time quantitative polymerase chain reaction (qPCR) and immunohistochemistry. Placental histopathology was evaluated. Clinical data was abstracted. Fisher’s exact test, Pearson correlations, and linear regression models were used to examine proportions and continuous data. 
between patients with active (<10 days since diagnosis) versus recovered COVID-19
(>10 days since diagnosis) at time of delivery. Secondary regression models adjusted for
labor status as a covariate and evaluated potential correlation between placental innate
immune gene expression and other variables.

Results: SARS-CoV-2 vRNA was detected in placental tissues from 5 women with
COVID-19 and from no controls (0/24, 0%). Only 1 of 5 cases with detectable SARS-CoV-
2 vRNA in placental tissues was confirmed to express SARS-CoV-2 nucleocapsid and
spike proteins in syncytiotrophoblast cells. We detected a significantly lower gene
expression of five critical innate immune mediators (IFNB, IFIT1, MXA, IL6, IL1B) in CV
and CAM from women with active or recovered COVID-19 compared to controls, which
remained significant after adjustment for labor status. There were minimal correlations
between placental gene expression and other studied variables including: gestational age
at diagnosis, time interval from COVID-19 diagnosis and delivery, pre-pregnancy body
mass index, COVID-19 disease severity or placental pathology.

Conclusion: A maternal SARS-CoV-2 infection was associated with an impaired
placental innate immune response in chorionic villous tissues and chorioamniotic
membranes that was not correlated with gestational age at COVID-19 diagnosis, timer
interval from COVID-19 diagnosis to delivery, maternal obesity, disease severity or
placental pathology.
Introduction

Pregnant women who become infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are more susceptible to hospitalization, critical illness, and numerous adverse perinatal complications (e.g., stillbirth, preeclampsia, preterm birth) and are associated with a higher likelihood of requiring ICU admission and mechanical ventilation as well as higher mortality rates.\(^1\)-\(^10\) The impact of a SARS-CoV-2 infection at the maternal-fetal interface and on the fetus, however, is not well understood.\(^11,12\) SARS-CoV-2 tropism for placental tissues is suggested to be low: the viral receptor necessary for cellular integration, angiotensin 2 converting enzyme, and its cofactor, transmembrane serine protease 2, are minimally expressed in healthy placental tissues.\(^13\)-\(^18\) Expression of these canonical receptors is increased, however, in syncytiotrophoblast cells from third trimester placentas of pregnant women with severe COVID-19 disease.\(^16,19\)-\(^22\) Numerous case series indicate that vertical transmission of SARS-CoV-2 to the fetus is low.\(^9\),\(^20\),\(^23\) How the placenta responds to a SARS-CoV-2 infection in pregnancy is unclear; an antiviral immune response has significant implications for both placental and fetal health.

Antiviral innate immunity primarily relies upon the synthesis and secretion of type I interferons (IFN), such as IFN-α and IFN-β, which further stimulate the production of hundreds to thousands of IFN stimulated genes, cytokines, and chemokines.\(^24\) SARS-CoV-2 is known to evade antiviral innate immunity through impairing placental innate immunity and gene expression during an acute infection. Viral non-structural protein 1 (Nsp1) and open reading frame 6 (ORF6) inhibit phosphorylation of signal transducer and activator-of-transcription (STAT) \(^25\) proteins and impede mRNA production or processing
while promoting host mRNA destruction.\textsuperscript{25-27} Impaired STAT protein phosphorylation by SARS-CoV-2 suppresses IFN expression and signaling, broadly evading innate immune responses.\textsuperscript{27, 28} To what extent a placental infection by SARS-CoV-2 modulates the placental antiviral innate immune response is unclear.\textsuperscript{29-34} A robust or dysregulated innate immune response may increase risk for inflammatory tissue injury or placental compromise and contribute to deleterious fetal outcomes including stillbirth.\textsuperscript{11, 35-40} A spectrum of placental pathology has been linked to a maternal SARS-CoV-2 infection including chronic histiocytic intervillositis, fibrin deposition, trophoblast necrosis and, in some cases, chronic villitis or acute chorioamnionitis; notably, stillbirth cases have been closely associated with the triad of chronic histiocytic intervillositis, fibrin deposition and trophoblast necrosis.\textsuperscript{9, 19, 41-48} Prior reports of placental pathology associated with COVID-19 have attributed these changes to maternal hypoxia from underlying respiratory impairment; whether an interferon and/or cytokine response to SARS-CoV-2 infection may contribute to a placental pathologic profile is unclear. Moreover, whether maternal disease status impacts the relationship between a SARS-CoV-2 infection, placental innate immune response, and placental histopathology is unknown.

The study objective was to evaluate the profile of the placental antiviral innate immune response following maternal COVID-19 disease in a large placental biobank that allowed for analysis of factors that are typically not explored due to smaller sample sizes (i.e., labor status, placental pathology, time interval between infection and delivery, and COVID-19 disease severity). We hypothesized that the placental antiviral response might be upregulated by a recent SARS-CoV-2 infection in pregnancy; however, a SARS-CoV-
2 infection may also harm cytotrophoblast and syncytiotrophoblast cells later in the disease course to impair the placental antiviral immune response. Thus, the placental antiviral innate immune response may clear SARS-CoV-2 at the expense of placental cellular health and immune defense.

Materials and Methods

Study Design, Sample Collection and Medical Record Abstraction

We conducted a case-control study that included placental tissues from pregnant patients with (N=140) and without (N=24) a positive laboratory test for SARS-CoV-2 by polymerase chain reaction of a nasopharyngeal swab during their pregnancy between 06/2020 – 07/2021. Placental tissues were collected with approval through either the Intermountain Healthcare Research Institutional Review Board (IRB # 1051448, waiver of consent) or the University of Washington Human Subjects Division (STUDY #0002410, informed consent). Coded placental samples and data from Intermountain Healthcare were sent to the University of Washington. As Dr. Adams Waldorf and her team did not have access to any personal identifiers linked to the Intermountain Healthcare samples, this study was deemed to not involve human subjects activity by the Human Subjects Division of the University of Washington (STUDY #00012244).

Placental tissues were collected by medical providers at the time of delivery. Placental tissue samples (chorioamniotic membranes, CAM; chorionic villous tissues, CV) were stored immediately in RNALater (Invitrogen, Waltham, MA) or in 10% neutral buffered formalin (CAM, CV, umbilical cord). The tissues in RNALater were subsequently
transferred to –80°C. Tissues in formalin were embedded in paraffin so that a cross-
section of each tissue could be evaluated.

Clinical data was abstracted from patient charts including pre-pregnancy body mass index
(BMI, kg/m²), parity, gestational age at COVID-19 diagnosis and delivery, severity of
COVID-19 infection, mode of delivery, pregnancy-related complications, neonatal sex
(University of Washington patients only), birthweight and COVID-19 infection status of the
neonate at 72 hours. Active COVID-19 and recovered COVID-19 were defined as delivery
≤10 or >10 days, respectively, from symptom onset or diagnosis; this categorization was
independent of disease severity. At the time of placental collection, 51 patients had active
COVID-19 and 89 had recovered COVID-19. We employed criteria for COVID-19 disease
severity previously defined in nonpregnant adults and adjusted them to our pregnant
cohort.49, 50 Categories for COVID-19 disease severity were scored as (0) for
asymptomatic disease, (1) for mild disease and (2) for moderate/severe disease. COVID-
19 disease severity was defined as asymptomatic disease (no reported symptoms), mild
(pneumonia symptoms of fever, cough, sore throat, muscle pain without shortness of
breath), moderate (dyspnea, respiratory rate ≥30 breaths/min, percutaneous oxygen
saturation ≤93% on room air at rest, arterial oxygen tension over inspiratory oxygen
fraction of less than 300 mmHg, and/or lung infiltrates >50% within 24 to 48 hours), and
severe (severe respiratory distress, respiratory failure requiring mechanical ventilation,
shock, and/or multiple organ dysfunction or failure). All laboratory assays, histopathology
analysis and statistical analysis were performed at the University of Washington.
placental samples and associated clinical metadata abstracted from Intermountain Healthcare were sent to the University of Washington.

Quantitative Real-Time Polymerase Chain Reaction

We performed quantitative real-time polymerase chain reaction (qPCR for SARS-CoV-2 viral RNA (vRNA) and a panel of innate immune genes from 164 placentas (N=140 with COVID-19; N=24 uninfected controls). First, placental samples were homogenized in TRIzol and then vRNA was extracted using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s protocol. RNA concentrations were measured by nanodrop, which reported A260/A280 ratios that confirmed acceptable purity without significant DNA or protein contamination. Next, reverse transcription and cDNA synthesis was performed for qPCR detection of SARS-CoV-2 using the absolute quantification (standard curve) method and the 2019-nCoV RUO kit (Integrated DNA Technologies, Inc., Coralville, IA). These reactions were run in duplex with the housekeeping gene TBP (TATA box binding protein). Reaction mixtures were made using the Taqman Fast Advance Master Mix (cat# 4444964, Thermo Fisher Scientific, USA), SARS-CoV-2 N2 primers and probes from the 2019-nCoV RUO kit (10006713, Integrated DNA Technologies MNC., USA) with an added quencher, TBP primers and probe, and nuclease free water. Viral load standards, positive control, no treatment control, and 400ng of supernatant RNA samples were pipetted into a 96 well plate in triplicate and mixed with master mix. Plates were then read by a QuantStudio 3 (ThermoFisher, Inc., Waltham, MA). Run conditions were 2 minutes at 50°C, 2 minutes at 95°C, then cycled 40 times at 1 second at 95°C and 20 seconds at 60°C. An internal ZEN quencher was
added to the 2019-nCoV_N2 probe to improve sensitivity. The sequence of this probe was 5'-FAM-ACAATTTGC/ZEN/CCCAGCGCTTCAG-3IABkF-3'. The N2 forward primer sequence was 5'-TTACAAACATTGGCCGCAA-3'. The N2 reverse primer sequence was 5'-GCGCGACATTCCGAAGAA-3'. The TBP probe sequence was: 5'-Quasar670-CACAGGAGCCAAAGGTGAAGAACAGT-BHQ-BHQ-3'. The TBP Primer/Probe was obtained from ThermoFisher (catalog# Hs00427620_m1; ThermoFisher Scientific, Waltham, MA). TBP amplification was performed in duplex with SARS-CoV-2 N2 for two reasons: 1) TBP amplification served as a quality control for the SARS-CoV-2 qPCR to ensure that each well with a negative result contained RNA; and 2) TBP is a housekeeping gene against which we performed comparative $2^{\Delta\Delta CT}$ qPCR to quantify antiviral IFN, IFN-stimulated gene (ISG) and cytokine gene expression. We used the Taqman assay platform to quantify a panel of Type I IFN (IFNA2, IFNB), ISG (MXA, IFIT1) and cytokines (IL6, IL1B). Primer assay IDs were: IFNA2, Hs00265051_s1; IFNB, Hs01077958_s1; MXA, Hs00895609_m1; ifit1, Hs01675197_m1; IL6, Hs00174131_m1; and IL1B, Hs01555410_m1 (Applied Biosystems, Waltham, MA). After reverse transcription was completed, qPCR was carried out on a QuantStudio 3 Real-Time PCR system (ThermoFisher, Inc.).

**Placental Histopathology**

Formalin-fixed placental samples were available and evaluated from 148 of 164 subjects. For each placenta, a hematoxylin-and-eosin-stained section was evaluated for chorioamnionitis, villous or intervillous inflammation, intervillous fibrin deposition, necrosis, or other lesions by a board-certified pediatric pathologist (RPK), who was
blinded to the patient’s COVID-19 status. We focused analysis of placental pathology on key features linked to SARS-CoV-2 infection: chronic histiocytic intervillitis, perivillous fibrin deposition and trophoblast necrosis. Villitis and other forms of chorionic villous pathology were staged as focal (a solitary group of involved contiguous villi) or multifocal.

**Immunohistochemistry**

SARS-CoV2 immunohistochemistry was performed on 5 µm-thick paraffin sections from formalin-fixed placental samples using a Ventana Benchmark II automated immunostainer and two different rabbit polyclonal antibodies specific for the nucleocapsid (Sino Biological, cat# 40143-R001) and spike (Sino Biological, cat# 40150-T62-CoV2-Spike) proteins, respectively. The nucleocapsid antibody was used at a 1:800 dilution with 68 min of citrate-based heat-induced enzyme retrieval (pH ~6), 20 min incubation time at 37°C and avidin-biotin blocking. The spike protein antibody was used at 1:500 dilution with mild CC1 conditioning, 32-minute incubation time at 37°C, and avidin-biotin blocking. A positive immunostaining control was a SARS-CoV-2 PCR-positive pregnant patient; this patient delivered a stillborn infant that was not included in this study. Two negative immunostaining controls were performed. The first negative control represented omission of the primary nucleocapsid or spike protein antibody from the positive control. Another negative immunostaining control came from a healthy, uninfected pregnancy.

**Statistical Analysis**

Demographic and SARS-CoV-2 infection characteristics in pregnancy were summarized by proportions and means. All analyses were conducted in ‘R’ software. To examine
relationships between discrete variables we used Fisher’s exact test and Pearson correlations for relationships between continuous variables. Comparisons were made between distinct placental tissue samples taken at delivery; there was no analysis of repeated measures. Differences in gene expression were examined using linear regression models with binary indicator variables for the active and recovered COVID-19 groups, which implicitly compare each with the control group. This results in two independent predictors in the model with no need to adjust p-values for multiple tests among the diagnostic groups. A second regression model was run in which labored status (labored vs. non-labored) was added as a covariate. A p-value <0.05 was considered statistically significant. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Results**

A total of 164 pregnant individuals were studied, consisting of 24 uninfected healthy controls and 140 individuals with COVID-19 disease. Clinical characteristics, COVID-19 disease and maternal-neonatal outcomes for these subjects are shown in Table 1. Pregnant individuals with or without COVID-19 were typically healthy with few comorbidities, multiparous, and overweight prior to pregnancy (Table 1). The mean gestational age at delivery was similar between pregnant women with COVID-19 compared to healthy controls (COVID-19: 37.8 ± 2.4 vs. Controls: 38.5 ± 1.2; t=-2.00, p=0.05). A greater proportion of individuals with COVID-19 labored prior to delivery compared to healthy controls (80.0% vs 50.0%, p=0.004). Preterm birth (<37 weeks) occurred in 21 of 140 pregnant women with COVID-19 (15.0%, p=0.2) and in 1 of 24
controls (4.2%, p=0.203). Fetal birth weight was significantly lower in the COVID-19 group (3,111.3 ± 610.0 vs 3,421.0 ± 496.3, t=-2.73, p=0.010). Apgar scores at 1 minute were significantly lower in the COVID-19 group (COVID-19: 7.63 ± 1.22 vs. Control: 7.9 ± 0.31; p=0.037), but Apgar scores at 5 minutes were similar for both groups. The mean gestational age at COVID-19 diagnosis was 30.6 ± 8.1 and the diagnosis to delivery interval was 7.2 ± 8.0 (range: 0 – 33 weeks). Out of 140 individuals with a history of COVID-19 diagnosed in pregnancy, 36% (N=51) had active COVID-19 and 64% (N=89) had recovered COVID-19 at delivery. Among the pregnant women diagnosed with COVID-19, disease severity was reported as asymptomatic in 24% (N=34), mild in 51% (N=71), moderate/severe in 10% (N=14) and unknown in 15% (N=21); no patients died.

Pathologic findings from cases and controls with SARS-CoV-2 associated pathology are shown in Table 2. Next, we quantified SARS-CoV-2 viral load in placental tissues to determine whether viral load was associated with placental pathology: SARS-CoV-2 vRNA was detected in placental tissues from 5 women with a history of COVID-19 in pregnancy (5/140, 3.6%), and in no controls (0/24, 0%). SARS-CoV-2 vRNA was detected in either CV (N=2) or CAM (N=2) tissues, or both CV and CAM (N=1). We performed immunohistochemistry for the SARS-CoV-2 nucleocapsid and spike proteins to determine whether SARS-CoV-2 antigens could be detected within these 5 tissues with detectable SARS-CoV-2 vRNA by qPCR, as well as other vRNA-negative tissues from patients with COVID-19 (N=7) and uninfected healthy controls (N=5, Figure 1). We found that only 1 of 5 cases with detectable SARS-CoV-2 vRNA in the placental tissues was confirmed to express SARS-CoV-2 nucleocapsid (Fig. 1E-G) and spike (Fig. 1I-L) proteins in
syncytiotrophoblast cells. In this case of active COVID-19, the viral load in CV tissues was high ($1.2 \times 10^{11}$ copies/mg) and associated with widespread placental basal infarcts, trophoblast necrosis, chronic villitis and diffuse perivillous fibrin deposition with histiocytic intervillosous inflammation (Fig. 1B-D). Of significance, this patient experienced preterm premature rupture of membranes (PPROM) and delivered a preterm infant at 33 weeks gestation, six days after COVID-19 diagnosis. Overall, histiocytic chorionic villitis and/or perivillous fibrin deposition was more frequent in placental tissues with detectable SARS-CoV-2 vRNA (2/5, 40%) than in the COVID-19 group without detectable SARS-CoV-2 vRNA (13/128, 10%) and in uninfected controls (3/20, 15%), however, these differences in proportion across groups were not significant (p=0.09).

Next, we evaluated placental expression of a panel of antiviral innate immune genes representative of the Type I IFN (IFNA2, IFNB), ISG (IFIT1, MXA) and the NF-κB cytokine (IL6) and Interleukin-1β (IL1B) cytokine response (Fig. 2). These genes represent key modulators of antiviral innate immunity: IFN-β, IL1β, and IFN-α2 coordinate inflammatory and antiviral immune actions, MxA and IFIT1 inhibit viral replication, and IL-6 coordinates broad inflammatory and immune responses. Pregnant women with recovered COVID-19 in CAM (Fig. 2) and CV (Fig. 2) tissues had significantly diminished expression of IFNB, IFIT1, MXA, IFNB, IL6 and IL1B (all, p<0.05). Interestingly, the case with detectable SARS-CoV-2 vRNA in CAM tissues 17 weeks after COVID-19 diagnosis had a very high IL6 gene expression compared to the housekeeping gene (8.3-fold, Fig. 2, triangle in recovered COVID-19 group). Pregnant individuals with active COVID-19 had a similar profile of significantly diminished innate immune gene expression in the placental
tissues (CAM: *IFIT1, MXA, IL6, IL1B*; CV: *IFNB, IFIT1, MXA, IL6, IL1B*; all, p<0.05). In
contrast, gene expression of *IFNA2* was similar between healthy controls and COVID-19
groups except for significantly diminished gene expression in the CV active COVID-19
group (p<0.05). Notably, we tested the gene expression distributions for stochastic
ordering in each target gene among all instances in which the active or recovered COVID-
19 group showed a significant mean difference relative to controls. In each case, there
was evidence for significant stochastic ordering (p-values <0.01), providing further
support for altered placental innate immune gene expression in pregnant women with
COVID-19. In summary, both an active and recovered maternal SARS-CoV-2 infection
was associated with diminished gene expression for a range of antiviral innate immune
signaling proteins. To determine if the inflammatory process of labor affected placental
gene expression between the COVID-19 and uninfected control groups, we added labor
status (whether a patient did or did not experience labor before delivery) as a covariate
to analyses of gene expression (Tables 3-8). Labored tissues had consistently lower
expression of *IFNB, IFIT1, MXA* and *IL1B*, but greater *IL6* expression. Upon adjusting for
labor status, multiple linear regression analyses determined that the patterns of gene
expression across controls and COVID-19 groups did not change substantially in either
CAM or CV tissues (Tables 3-8). Across 24 comparisons - two tissues (CV and CAM), 2
groups (active and recovered), and 6 genes (*IFNA2, IFNB, IFIT1, MXA, IL6, IL1B*) – there
were 20 significant differences between either active or recovered COVID-19 and the
corresponding control group. After adjustment for labor, 18 significant differences
remained with the loss of *IFIT1* (active COVID-19 vs. controls in CV; p=0.08; Table 5) and
*IL1B* (active COVID-19 vs. controls in CAM; p=0.08; Table 8). In summary, labor status
did not substantially impact the gene expression profile in placental tissues or alter the findings of diminished innate immune gene expression.

We also correlated placental immune gene expression with gestational age at COVID-19 diagnosis (Fig. 3), COVID-19 disease severity (Fig. 4), maternal pre-pregnancy body mass index (BMI; Fig. 5), time interval between COVID-19 diagnosis and delivery (Fig. 6) and detection of SARS-CoV-2-associated placental pathology (Fig. 6). With few exceptions, the line of best fit was flat for both CV and CAM tissues with no consistent correlations between innate immune gene expression and either gestational age at COVID-19 diagnosis, COVID-19 disease severity, maternal BMI, time interval between diagnosis and delivery or placental pathology. Gene expression of IFNA2 in unlabored CAM tissues was significantly negatively correlated with time interval from COVID-19 diagnosis, such that a longer interval was associated with lower expression (p=0.04; Fig. 7). A significant positive correlation was identified between SARS-CoV-2-associated placental pathology and IL6 and IL1B gene expression in unlabored CAM tissues indicating that higher IL6 and IL1B expression was more likely when pathology was observed (IL6: p=0.004, IL1B: p=0.02; Fig. 6). Overall, placental antiviral innate immune gene expression did not correlate with COVID-19 disease severity or gestational age at infection. In summary, there were very few significant correlations between gene expression, placental pathology and time interval between infection and delivery.

**Comment**

*Principal Findings*
Our data indicate that a maternal SARS-CoV-2 infection leaves the placenta with a diminished innate immune response in both the CV tissues in the placental disc and the CAM. Consistent with other studies, detection of SARS-CoV-2 vRNA in placental tissues was infrequent and the presence of SARS-CoV-2 antigens could only be confirmed in a single case. Despite rare detection of SARS-CoV-2 proteins within the placenta, there was a consistently lower expression of critical Type I IFN, ISG and cytokines that direct the antiviral immune response in placental tissues from pregnant women with recovered and active COVID-19 disease. Notably, our findings remained significant after controlling for labor status, a known confounder due to inflammatory processes occurring during labor and delivery.\textsuperscript{54, 55} Interestingly, there were very few (or no) correlations between placental gene expression and other studied variables including: gestational age at diagnosis, time interval from COVID-19 diagnosis and delivery, pre-pregnancy body mass index, COVID-19 disease severity or placental pathology. This finding is important because it means that pregnant people with even a mild COVID-19 disease course at any time in pregnancy and of any body habitus are equally susceptible to SARS-CoV-2 placental innate immune suppression. Whether an impaired placental immune response correlates with other vital functions and might underlie the increased stillbirth risk associated with maternal COVID-19 is unknown.

\textit{Results in the Context of What is Known}

Interestingly, our findings parallel that of other studies in mice indicating “viral priming”, by which an initial viral infection impairs placental and cervical immunity thereby increasing risk of subsequent infection. In murine models of MHV-68 (a murine
herpesvirus) infection, a subclinical viral infection sensitized maternal immune responses to co-infection or subsequent infection by other microorganisms.\textsuperscript{56, 57} Viral infections in these models compromised innate immune responses in cervical tissues, predisposing to intrauterine bacterial infections. MHV-68 diminished inflammatory and immune responses through decreased toll-like receptor gene expression and subsequent downregulation of cytokine and chemokine gene expression that altered proinflammatory responses against bacterial pathogens.\textsuperscript{56, 57}

\textit{Research Implications}

Our results present several important research questions and generate new hypotheses. First, we hypothesize that impaired innate immunity is only one aspect of placental function that is impaired following a SARS-CoV-2 infection, likely due to the placental host response to control the infection. Certainly, histopathologic evidence of placental injury is well documented to occur in some cases of COVID-19 that manifest as SARS-CoV-2 placentitis, chronic histiocytic villitis, intervillous fibrin deposition, trophoblast necrosis, maternal vascular malperfusion and deposition of intervillous thrombi.\textsuperscript{40, 58, 59} As the canonical receptors for SARS-CoV-2 are not typically co-expressed in the placenta and viral infection of syncytiotrophoblast cells appears rare, it is more probable that innate immune mediators released during an acute infection are the source of placental injury than a primary viral infection itself.\textsuperscript{20, 60} Regardless of the mechanism of placental injury, an impairment of placental immune functioning is likely to parallel other defects in metabolic and biological pathways that should be defined in order to understand SARS-CoV-2 pathogenesis in the placenta. Interestingly, several reports have highlighted a
reduction in SARS-CoV-2 antibody transfer after a natural SARS-CoV-2 infection, which may be due to an impairment in placental function.\textsuperscript{20,61,62}

Whether the placental and decidual immune response to SARS-CoV-2 is activated or impaired likely depends on the tissue and cell-type studied, as well as the time course. Two studies employing single cell RNA-Seq analysis of pregnant individuals with mild (N=9) and severe (N=2) COVID-19 identified a broad activation of myeloid cells in the decidua of pregnancies with COVID-19.\textsuperscript{16} In pregnant individuals with mild COVID-19, there was also evidence for enrichment of decidual IL-1\(\beta\)-producing macrophages and an attenuation of interferon signaling in the decidua.\textsuperscript{63} As we focused investigation on the CV and CAM tissues, and not the maternal decidua, it is unclear if immune activation is spatially restricted to certain decidual subsets, while innate immunity becomes quickly attenuated in the CV and CAM following infection. Finally, the temporal course of the SARS-CoV-2 innate immune response in maternal decidua and human placentas is not well defined, as most samples have been collected at birth days to weeks and not hours after the initial diagnosis, including the ones in our cohort.

Our findings also underscore that a positive SARS-CoV-2 PCR in the placenta may not correlate with antigen positivity by immunohistochemistry. This may occur for several reasons. Viral antigen positivity can be patchy, depending on where the viral infection occurred in the placenta. A placental biopsy for PCR studies might have sampled an infected area, but a second biopsy preserved in formalin for immunohistochemistry could have missed a virally-infected area. Stereotactic biopsies of the placenta are not always
possible in clinical research, especially due to COVID-19 pandemic restrictions on the entry of laboratory personnel into clinical areas. Clearance of viral antigens also tends to precede the clearance of viral RNA. Finally, SARS-CoV-2 genomic RNA remains for days to weeks after viral entry and avoids degradation by cellular nucleases. Infection is highly variable between cells and only small cell populations will have a high burden of SARS-CoV-2 RNA. It is possible that adjacent placental biopsies from a patient with a SARS-CoV-2 infection may not be concordant in their expression of viral RNA and protein.

Clinical Implications

This data has several implications for clinical care. First, our observations that the placental innate immune response was impaired regardless of disease severity suggest that even a mild COVID-19 disease course can impair innate immunity in the CAM and CV. Whether an impaired placental innate immune response increases a pregnant individual’s susceptibility to chorioamnionitis is unknown. A meta-analysis of approximately 1,500 pregnancies with COVID-19 revealed a higher-than-expected rate of chorioamnionitis (26%) compared to historical published studies from unexposed placentas (4-20%). Large studies evaluating rates of chorioamnionitis after a natural COVID-19 infection are needed to determine if rates are higher than expected in an uninfected population.

Although there are many studies of the impact of COVID-19 on obstetrical and neonatal outcomes, the long-term neurodevelopmental and neuropsychiatric health
among children born to mothers with a SARS-CoV-2 infection during pregnancy is unclear. The “maternal immune activation” hypothesis proposes that fetal exposure to inflammation can adversely impact fetal neurodevelopment and increase the risk for neuropsychiatric and developmental disorders. A large body of literature supports a link between diverse maternal infections and abnormal fetal neurodevelopment. Even a maternal fever has been associated with an increased risk of autism spectrum disorder in the child in the Norwegian Mother and Child Cohort Study (114,500 pregnant people).

Although the pathogenesis linking maternal immune activation to aberrant fetal neurodevelopment is not well defined, many of the possible links involve placental injury or inflammation.

There is early evidence that exposure to a maternal SARS-CoV-2 infection in utero might be associated with a higher rate of neurodevelopmental diagnoses. A retrospective cohort study of infants born to pregnant individuals with SARS-CoV-2 infections during pregnancy found that there was a significantly greater odds of having a neurodevelopmental diagnosis in infants exposed to a maternal SARS-CoV-2 infection (14/222; 6.3%) versus a healthy pregnant control group (227/7550; 3.0%) in the first 12 months of life. This study was limited by cohort size, one year follow-up and a broad inclusion of developmental diagnoses; nevertheless, this is important early evidence that the motor and cognitive development of these children should be followed closely.

Longitudinal studies of children exposed to COVID-19 in utero should continue over a period of 30 years to determine if there is a higher rate of neurodevelopmental or neuropsychiatric diagnoses compared to children from uninfected pregnancies. Based on
our prior study using the Swedish population-based birth registry, we predict that at least
7 years of follow-up of a large population will be needed to determine differences in rates
of autism spectrum disorder and 25-30 years of follow-up to evaluate differences in rates
of psychosis and schizophrenia.\textsuperscript{73}

We recognize the enormity of the public health challenge to survey the neurodevelopment
of all children exposed to SARS-CoV-2 \textit{in utero}. As the COVID-19 pandemic continues
to evolve and transition into an endemic phase, it is likely that hundreds of thousands of
infants would need to be followed over a period of at least 7 years. The public health
surveillance of pregnancy and neonatal outcomes in the U.S. is simply inadequate for this
task. Many European countries that have a more robust public health infrastructure are
better prepared for such a challenge. Nevertheless, standardized assessments of
neurodevelopment could be performed for children exposed to SARS-CoV-2 at regular
intervals, matching the well-child visit schedule. The infrastructure and methods
developed during and following the Zika virus (ZIKV) epidemic in 2014-2016 may provide
a useful toolkit for investigators to follow the neurodevelopment of children exposed to
SARS-CoV-2 \textit{in utero}.\textsuperscript{85-88} Importantly, the adverse impact of fetal exposure to infectious
diseases can impact neurodevelopment and mental health through adolescence and into
adulthood.\textsuperscript{72, 73} We predict that the sequelae of COVID-19 disease in pregnancy will
continue for decades, manifested in higher risks of neuropsychiatric disease in the
exposed children.

\textbf{Strengths and Limitations}
There are several reports highlighting placental injury and inflammation following a SARS-CoV-2 infection in pregnancy, but the relationships between placental immunity, histopathology, labor status, COVID-19 disease severity, duration of infection, and gestational age at diagnosis were not addressed. Our study is the first to employ a large placental biobank to correlate these variables with markers of innate immunity and evaluate how the placental antiviral immune response is impacted by a maternal COVID-19 infection. Our study assessed the expression of a broad range of placental innate immune genes, including the Type I IFN, ISG and cytokine response, providing insight into placental innate immunity not previously described.

Study limitations include the lack of specimens collected within the first day or hours of a SARS-CoV-2 infection, which is also typical in other studies. This limits our ability to assess whether an acute infection might upregulate the innate immune response prior to a decrease in innate immune mediators. Secondly, we likely underestimated SARS-CoV-2 placental pathology, as we only collected and evaluated a single CV biopsy or CAM roll per placenta. Placental pathology can also be “patchy”, and it is possible that we missed pathology that was present in unsampled areas of the placenta. We also note that one subject delivered twins; our clinical data does not reveal whether one or two placentas were sampled from this unique patient.

**Conclusions**

In summary, we determined that a maternal SARS-CoV-2 infection can significantly impair the antiviral innate immune response in placental tissues, with sustained immune
suppression for weeks to months, regardless of disease severity or gestational age at infection. We are particularly concerned that this impact on the placenta was observed regardless of the severity of the COVID-19 disease course, the timepoint in pregnancy at which SARS-CoV-2 was contracted, and the maternal body habitus. Our results highlight the need for further study of immune regulation following SARS-CoV-2 infection in placental tissue and susceptibility to infection. Evaluating the placental capacity for immune response and metabolic function after COVID-19 will be imperative to understanding risks for stillbirth, chorioamnionitis and other adverse health outcomes for the child.\textsuperscript{12,21}
Acknowledgements

We thank the pregnant patients at Intermountain Health Care for providing the placental tissues that made this study possible, as well as the surgical technicians and nurses at the University of Utah, who enabled this research. This work was supported primarily by funding from charitable donations and the National Institutes of Health AI143265. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or other funders. Our use of the term ‘women’ is meant to be trans-inclusionary, acknowledging that some biologically born females identify as non-binary or transgender and some biologically born males identify as women, transgender, or as non-binary.

Author Contributions

The manuscript was written by B.C., K.A.W, T-Y.W., J.M. and O.C. Placental tissue samples were collected under the direction of H.F., L.C, S.E. and K.A.W. Placental histopathology was reviewed and scored by R.P.K. All authors provided critical edits to the manuscript. Experiments were performed by B.C., T-Y.W., N.B., M.D. and O.C. Statistical analyses were performed by J.M.
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Table 1. Clinical Characteristics, COVID-19 Disease and Pregnancy and Neonatal Outcomes

<table>
<thead>
<tr>
<th></th>
<th>All (N=164)</th>
<th>Pregnant Individuals</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Controls (N=24)</td>
<td>COVID-19 (N=140)</td>
</tr>
<tr>
<td><strong>Pregnancy and Medical History</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>2.6 ± 1.6</td>
<td>2.5 ± 1.3</td>
<td>2.6 ± 1.6</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>28.6 ± 6.6</td>
<td>27.6 ± 6.7</td>
<td>28.7 ± 6.6</td>
</tr>
<tr>
<td>Obesity (BMI ≥30.0 kg/m²)</td>
<td>58 (35.4%)</td>
<td>6 (25.0%)</td>
<td>52 (37.1%)</td>
</tr>
<tr>
<td>Asthma</td>
<td>3 (1.8%)</td>
<td>0 (0%)</td>
<td>3 (2.1%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (1.2%)</td>
<td>1 (0.6%)</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>8 (4.9%)</td>
<td>3 (12.5%)</td>
<td>5 (3.6%)</td>
</tr>
<tr>
<td><strong>Pregnancy and Neonatal Outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery (weeks, N=162)</td>
<td>37.9 ± 2.2</td>
<td>38.5 ± 1.2</td>
<td>37.8 ± 2.4</td>
</tr>
<tr>
<td>Preterm birth rate</td>
<td>22 (13.4%)</td>
<td>1 (4.2%)</td>
<td>21 (15.0%)</td>
</tr>
<tr>
<td>PPROM</td>
<td>8 (4.9%)</td>
<td>0 (0%)</td>
<td>8 (5.7%)</td>
</tr>
<tr>
<td>Preeclampsia and gestational hypertension</td>
<td>19 (11.6%)</td>
<td>2 (8.3%)</td>
<td>17 (12.1%)</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>57 (34.8%)</td>
<td>12 (50.0%)</td>
<td>45 (32.1%)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>107(65.2%)</td>
<td>12 (50.0%)</td>
<td>95 (67.9%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>Labor</td>
<td>124(75.6%)</td>
<td>40 (24.4%)</td>
<td>12 (50.0%)</td>
</tr>
<tr>
<td>Fetal Birth Weight (g)</td>
<td>3,156.6 ± 603.5</td>
<td>3421.0 ± 496.3</td>
<td>3,111.3 ± 610</td>
</tr>
<tr>
<td>Apgar score at 1 min (N=154; 20 Control, 134 COVID-19)</td>
<td>7.67 ± 1.15</td>
<td>7.90 ± 0.31</td>
<td>7.63 ± 1.22</td>
</tr>
<tr>
<td>Apgar score at 5 min (N=154; 20 Control, 134 COVID-19)</td>
<td>8.75 ± 0.85</td>
<td>8.70 ± 0.57</td>
<td>8.76 ± 0.89</td>
</tr>
</tbody>
</table>

**COVID-19 Disease (N=140)**

<table>
<thead>
<tr>
<th>Gestational age at COVID-19 Diagnosis</th>
<th>-</th>
<th>-</th>
<th>30.6 ± 8.1</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19 diagnosis-to-delivery (weeks; N=138)</td>
<td>-</td>
<td>-</td>
<td>7.2 ± 8.0</td>
<td>-</td>
</tr>
<tr>
<td>COVID-19 status at delivery</td>
<td></td>
<td></td>
<td>51 (36.4%)</td>
<td>-</td>
</tr>
<tr>
<td>Active COVID-19</td>
<td>-</td>
<td>-</td>
<td>51 (36.4%)</td>
<td>-</td>
</tr>
<tr>
<td>Recovered COVID-19</td>
<td>-</td>
<td>-</td>
<td>89 (63.6%)</td>
<td>-</td>
</tr>
<tr>
<td>COVID-19 symptoms/severity</td>
<td></td>
<td></td>
<td>34 (24.3%)</td>
<td>-</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>-</td>
<td>34 (24.3%)</td>
<td>-</td>
</tr>
<tr>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>71 (50.7%)</td>
<td>-</td>
</tr>
<tr>
<td>Moderate/Severe</td>
<td>-</td>
<td>-</td>
<td>14 (10.0%)</td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td>-</td>
<td>-</td>
<td>21 (15.0%)</td>
<td>-</td>
</tr>
</tbody>
</table>
Abbreviations: BMI, body mass index; PPROM, preterm premature rupture of membranes; NS, non-significant. Information about maternal age and fetal sex was not available for most of the cohort and is, therefore, not reported.

* p-values are considered significant if <0.05.
Table 2. Cases and controls with SARS-CoV-2-associated placental pathology

<table>
<thead>
<tr>
<th>Case</th>
<th>Group</th>
<th>Placental Pathology Notes</th>
<th>SARS-CoV-2 vRNA Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COVID-19</td>
<td>Borderline increase in perivillous fibrin deposition without histiocytes encasing anchoring villi and adjacent villi</td>
<td>no</td>
</tr>
<tr>
<td>2</td>
<td>COVID-19</td>
<td>Multiple small foci of perivillous fibrin deposition with histiocytic intervillous inflammation, one focus with active villous inflammation</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>COVID-19</td>
<td>Multiple small foci of perivillous fibrin deposition with histiocytic intervillous inflammation</td>
<td>no</td>
</tr>
<tr>
<td>4</td>
<td>COVID-19</td>
<td>Small group of villi with chronic villitis</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>COVID-19</td>
<td>Multiple foci of perivillous fibrin deposition and probable villitis without histiocytic intervillous inflammation</td>
<td>yes</td>
</tr>
<tr>
<td>6</td>
<td>COVID-19</td>
<td>Single tiny focus of perivillous fibrin deposition with histiocytic intervillous inflammation</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>COVID-19</td>
<td>Fetal side of disc with single focus of perivillous fibrin deposition and histiocytic intervillous inflammation</td>
<td>no</td>
</tr>
<tr>
<td>Case</td>
<td>Diagnosis and Description</td>
<td>SARS-CoV-2 Association</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>------------------------------------------------------------------------------------------</td>
<td>------------------------</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Single tiny focus of chronic villitis</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Focal chronic villitis</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Diffuse perivillous fibrin deposition with histiocytic intervillous inflammation</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Basal zone of perivillous fibrin deposition with histiocytic intervillous inflammation</td>
<td>no</td>
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<tr>
<td>12</td>
<td>Widespread placental basal infarcts, trophoblast necrosis, chronic villitis and diffuse perivillous fibrin deposition with histiocytic intervillous inflammation</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Single minute focus of chronic villitis</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Borderline increase in perivillous fibrin deposition without histiocytes encasing anchoring villi and adjacent villi</td>
<td>no</td>
<td></td>
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<tr>
<td>15</td>
<td>Basal zone lymphohistiocytic villitis and fibrin deposition just along the decidual interface</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control Patchy perivillous fibrin deposition</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Control Patchy perivillous fibrin deposition; one focus of chronic villitis</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Control 2 tiny foci of chronic villitis</td>
<td>no</td>
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</tbody>
</table>

This table reflects notes from the placental pathologist (RPK) for each case that was considered to have chronic villitis or one or more feature of SARS-CoV-2-associated placental pathology (chronic histiocytic villitis, perivillous fibrin deposition and/or trophoblast necrosis). Cases were included even if they had a tiny focus of pathology as it was unclear whether these small foci.
might reflect similar pathology in other regions of the placenta, which were not sampled. SARS-CoV-2 vRNA was detected in 2 of the 15 COVID-19 cases with placental pathology and is shown here to allow for correlation with pathologic findings.
Table 3. Linear regression models of *Ifna2* gene expression in CAM and CV by COVID-19 disease status with and without adjustment for labor status.

<table>
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<tbody>
<tr>
<td><strong>Chorioamniotic Membranes</strong> (CTL: N=18; ACT: N=41; RECOV: N=68)</td>
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</tr>
<tr>
<td>CTL (Intercept)</td>
<td>-3.31</td>
<td>0.60</td>
<td>-5.49</td>
<td><em>&lt;0.001</em></td>
<td>-2.62</td>
<td>0.64</td>
<td>-4.08</td>
<td><em>&lt;0.001</em></td>
<td>-2.58</td>
<td>0.44</td>
<td>-5.84</td>
<td><em>&lt;0.001</em></td>
<td>-2.30</td>
<td>0.49</td>
<td>-4.69</td>
<td><em>&lt;0.001</em></td>
</tr>
<tr>
<td>ACT</td>
<td>-0.60</td>
<td>0.72</td>
<td>-0.83</td>
<td>0.407</td>
<td>-0.25</td>
<td>0.72</td>
<td>-0.34</td>
<td>0.731</td>
<td>-1.28</td>
<td>0.55</td>
<td>-2.31</td>
<td>0.022</td>
<td>-1.13</td>
<td>0.56</td>
<td>-2.01</td>
<td>0.047</td>
</tr>
<tr>
<td>RECOV</td>
<td>-0.72</td>
<td>0.68</td>
<td>-1.06</td>
<td>0.291</td>
<td>-0.33</td>
<td>0.68</td>
<td>-0.49</td>
<td>0.624</td>
<td>-0.51</td>
<td>0.50</td>
<td>-1.02</td>
<td>0.311</td>
<td>-0.36</td>
<td>0.51</td>
<td>-0.71</td>
<td>0.477</td>
</tr>
<tr>
<td>Labored (Yes)</td>
<td>-1.38</td>
<td>0.51</td>
<td>-2.70</td>
<td>0.008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.52</td>
<td>0.42</td>
<td>-1.26</td>
<td>0.211</td>
<td></td>
<td></td>
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<tr>
<td>Observations</td>
<td>127</td>
<td>127</td>
<td>146</td>
<td>146</td>
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</tr>
<tr>
<td>R² /</td>
<td>0.009 / -0.007</td>
<td>0.065 / 0.042</td>
<td>0.041 / 0.027</td>
<td>0.051 / 0.031</td>
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<tr>
<td>R² adjusted</td>
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</tr>
</tbody>
</table>
We report coefficient, coefficient standard errors, t-statistic, and p-values for each analysis of fold change in gene expression for active and recovered COVID-19 groups. Significant p-values are shown in bold text. Model 1 contrasts the active and recovered COVID-19 positive cohorts with the study’s controls. Model 2 adds labor as a covariate without any interaction terms. Abbreviations: CTL, controls without a history of COVID-19; ACT, active COVID-19; RECOV, recovered COVID-19; Coeff, coefficient; SE, standard error.
Table 4. Linear regression models of *IFNB* gene expression in CAM and CV by COVID-19 disease status with and without adjustment for labor status.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Chorioamniotic Membranes (CTL: N=18; ACT: N=41; RECOV: N=68)</th>
<th>Chorionic Villous Tissue (CTL: N=23; ACT: N=40; RECOV: N=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>CTL (Intercept)</td>
<td>-0.93   0.48  -1.95 0.053  -0.36  0.52  -0.69 0.489  -0.31  0.36  -0.88 0.381  -0.14  0.39  -0.36 0.716</td>
<td></td>
</tr>
<tr>
<td>ACT</td>
<td>-1.11   0.58  -1.91 0.058  -0.78  0.59  -1.33 0.186  -1.69  0.44  -3.83 &lt;0.001 -1.59  0.45  -3.51 0.001</td>
<td></td>
</tr>
<tr>
<td>RECOV</td>
<td>-1.66   0.55  -3.04 0.003  -1.33  0.55  -2.42 0.017  -1.24  0.41  -3.07 0.003  -1.15  0.42  -2.76 0.007</td>
<td></td>
</tr>
<tr>
<td>Labored (Yes)</td>
<td>1.14    0.44   -2.57 0.011</td>
<td>-0.34  0.33   -1.01 0.313</td>
</tr>
<tr>
<td>Observations</td>
<td>147     147     152    152</td>
<td></td>
</tr>
<tr>
<td>$R^2 / R^2$ adjusted</td>
<td>0.061 / 0.048</td>
<td>0.103 / 0.084</td>
</tr>
</tbody>
</table>

We report coefficient, coefficient standard errors, t-statistic, and p-values for each analysis of fold change in gene expression for active and recovered COVID-19 groups. Significant p-values are shown in bold text. Model 1 contrasts the active and recovered COVID-19 positive cohorts with the study’s controls. Model 2 adds labor as a covariate without any interaction terms.
Abbreviations: CTL, controls without a history of COVID-19; ACT, active COVID-19; RECOV, recovered COVID-19; Coeff, coefficient; SE, standard error.
Table 5. Linear regression models of *IFIT1* gene expression in CAM and CV by COVID-19 disease status with and without adjustment for labor status.

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</thead>
<tbody>
<tr>
<td>CTL (Intercept)</td>
<td>1.43</td>
<td>0.40</td>
<td>3.60</td>
<td><strong>&lt;0.001</strong></td>
<td>2.10</td>
<td>0.42</td>
<td>4.97</td>
<td><strong>&lt;0.001</strong></td>
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<td>0.38</td>
<td>0.77</td>
<td>0.441</td>
<td>0.60</td>
<td>0.41</td>
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<td>-3.55</td>
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<td><strong>0.007</strong></td>
<td>-1.03</td>
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<td>-2.21</td>
<td><strong>0.029</strong></td>
<td>-0.84</td>
<td>0.47</td>
<td>-1.77</td>
<td>0.078</td>
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<tr>
<td>RECOV</td>
<td>-2.33</td>
<td>0.45</td>
<td>-5.12</td>
<td><strong>&lt;0.001</strong></td>
<td>-1.91</td>
<td>0.45</td>
<td>-4.25</td>
<td><strong>&lt;0.001</strong></td>
<td>-1.58</td>
<td>0.43</td>
<td>-3.69</td>
<td><strong>&lt;0.001</strong></td>
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<td>0.44</td>
<td>-3.20</td>
<td><strong>0.002</strong></td>
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<tr>
<td>Labored (Yes)</td>
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<td></td>
<td>-1.35</td>
<td>0.37</td>
<td>-3.68</td>
<td><strong>&lt;0.001</strong></td>
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<td>-0.61</td>
<td>0.35</td>
<td>-1.73</td>
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<tr>
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<tr>
<td>R² / R² adjusted</td>
<td>0.150/0.139</td>
<td>0.221/0.206</td>
<td>0.086/0.073</td>
<td>0.104/0.086</td>
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</tbody>
</table>

We report coefficient, coefficient standard errors, t-statistic, and p-values for each analysis of fold change in gene expression for active and recovered COVID-19 groups. Significant p-values are shown in bold text. Model 1 contrasts the active and recovered...
COVID-19 positive cohorts with the study’s controls. Model 2 adds labor as a covariate without any interaction terms.

Abbreviations: CTL, controls without a history of COVID-19; ACT, active COVID-19; RECOV, recovered COVID-19; Coeff, coefficient; SE, standard error.
Table 6. Linear regression models of MXA gene expression in CAM and CV by COVID-19 disease status with and without adjustment for labor status.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Chorioamniotic Membranes</th>
<th>Chorionic Villous Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(CTL: N=18; ACT: N=41; RECOV: N=68)</td>
<td>(CTL: N=23; ACT: N=40; RECOV: N=83)</td>
</tr>
<tr>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 1</td>
</tr>
<tr>
<td>CTL (Intercept)</td>
<td>5.14</td>
<td>0.40</td>
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<tr>
<td>ACT</td>
<td>-1.87</td>
<td>0.49</td>
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<tr>
<td>RECOV</td>
<td>-2.49</td>
<td>0.46</td>
</tr>
<tr>
<td>Labored (Yes)</td>
<td>-1.37</td>
<td>0.36</td>
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<tr>
<td>Observations</td>
<td>155</td>
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<tr>
<td>R² /</td>
<td>0.165 / 0.154</td>
<td></td>
</tr>
<tr>
<td>R² adjusted</td>
<td></td>
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</table>
We report coefficient, coefficient standard errors, t-statistic, and p-values for each analysis of fold change in gene expression for active and recovered COVID-19 groups. Significant p-values are shown in bold text. Model 1 contrasts the active and recovered COVID-19 positive cohorts with the study's controls. Model 2 adds labor as a covariate without any interaction terms. Abbreviations: CTL, controls without a history of COVID-19; ACT, active COVID-19; RECOV, recovered COVID-19; Coeff, coefficient; SE, standard error.
Table 7. Linear regression models of *IL6* gene expression in CAM and CV by COVID-19 disease status with and without adjustment for labor status.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Chorioamniotic Membranes</th>
<th>Chorionic Villous Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(CTL: N=18; ACT: N=41; RECOV: N=68)</td>
<td>(CTL: N=23; ACT: N=40; RECOV: N=83)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 1</th>
<th>Model 2</th>
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<tbody>
<tr>
<td>CTL (Intercept)</td>
<td>2.00</td>
<td>0.50</td>
<td>3.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACT</td>
<td>-1.45</td>
<td>0.61</td>
<td>-2.38</td>
<td>0.019</td>
</tr>
<tr>
<td>RECOV</td>
<td>-1.57</td>
<td>0.57</td>
<td>-2.74</td>
<td>0.007</td>
</tr>
<tr>
<td>Labored (Yes)</td>
<td>1.69</td>
<td>0.46</td>
<td>3.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Observations</td>
<td>152</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² / R² adjusted</td>
<td>0.050 / 0.037</td>
<td></td>
<td>0.128 / 0.111</td>
<td></td>
</tr>
</tbody>
</table>

We report coefficient, coefficient standard errors, t-statistic, and p-values for each analysis of fold change in gene expression for active and recovered COVID-19 groups. Significant p-values are shown in bold text. Model 1 contrasts the active and recovered
COVID-19 positive cohorts with the study’s controls. Model 2 adds labor as a covariate without any interaction terms.

Abbreviations: CTL, controls without a history of COVID-19; ACT, active COVID-19; RECOV, recovered COVID-19; Coeff, coefficient; SE, standard error.
Table 8. Linear regression models of *IL1B* gene expression in CAM and CV by COVID-19 disease status with and without adjustment for labor status.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Chorioamniotic Membranes</th>
<th>Chorionic Villous Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(CTL: N=18; ACT: N=41; RECOV: N=68)</td>
<td>(CTL: N=23; ACT: N=40; RECOV: N=83)</td>
</tr>
<tr>
<td>CTL (Intercept)</td>
<td>6.04 0.59 10.25 &lt;0.001</td>
<td>3.01 0.49 6.10 &lt;0.001</td>
</tr>
<tr>
<td>ACT</td>
<td>-1.52 0.73 -2.08 0.039</td>
<td>-1.32 0.75 -1.76 0.080</td>
</tr>
<tr>
<td>RECOV</td>
<td>-1.84 0.67 -2.74 0.007</td>
<td>-1.61 0.70 -2.32 0.022</td>
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<tr>
<td>Labored (Yes)</td>
<td>-0.71 0.56 -1.27 0.206</td>
<td>0.12 0.47 0.26 0.798</td>
</tr>
<tr>
<td>Observations</td>
<td>142 152</td>
<td>142 152</td>
</tr>
<tr>
<td>R² / R² adjusted</td>
<td>0.052 / 0.038</td>
<td>0.063 / 0.042</td>
</tr>
</tbody>
</table>

We report coefficient, coefficient standard errors, t-statistic, and p-values for each analysis of fold change in gene expression for active and recovered COVID-19 groups. Significant p-values are shown in bold text. Model 1 contrasts the active and recovered COVID-19 positive cohorts with the study’s controls. Model 2 adds labor as a covariate without any interaction terms.
Abbreviations: CTL, controls without a history of COVID-19; ACT, active COVID-19; RECOV, recovered COVID-19; Coeff, coefficient; SE, standard error.
Figure Legends.

Figure 1. Placental Histopathology and SARS-CoV-2 Immunostaining. Representative fields of hematoxylin and eosin-stained sections of placental chorionic villi are shown in the upper row from a healthy uninfected control patient (a) and three pregnant individuals with a history of COVID-19 during the pregnancy (b-d). Placental pathology included perivillous fibrin deposition and intervillos histiocytes (arrows) with (d) or without (b, c) trophoblast necrosis. In the middle and lower rows, we show immunohistochemistry with antibodies specific for the SARS-CoV-2 nucleocapsid (middle row) and spike proteins (lower row). A positive control is shown in Fig. 1e and 1i from a SARS-CoV-2 PCR-positive pregnant patient, who delivered a stillborn infant that was not included in this study. Negative controls included omission of the primary antibody nucleocapsid or spike protein from the positive control (Fig. 1h for nucleocapsid) and SARS-CoV-2 immunostaining of tissues from a healthy, uninfected control (Fig. 1f, 1j). SARS-CoV-2 antigen staining was demonstrated in the placental syncytiotrophoblast of one of five pregnant patients from our study that had SARS-CoV-2 PCR-positive placental tissues (g, k, l). No immunostaining was observed when either primary antibody for nucleocapsid or spike proteins was omitted (h, nucleocapsid shown) or when the placenta of a healthy, uninfected control was stained for SARS-CoV-2 nucleocapsid or spike proteins (f, j). The pattern of labeling in the placenta from a subject with COVID-19 in our study (Fig. 1g, 1k-l) was like that observed in the positive control (e, i). Scale bars shown in panel ‘a’ reflect the magnification of panels a-d and the bar in panel ‘e’ applies to e-k.
**Figure 2.** Placental Innate Immune Gene Expression in Patients with and without COVID-19 in Pregnancy Stratified by Disease. The boxplots illustrate the relative innate immune gene expression in placental tissues compared to expression of the TATA-Box Binding Protein (*TBP*, housekeeping gene). Gene expression is shown in uninfected control pregnant women (blue) compared to pregnant patients with either active COVID-19 (red, PCR diagnosis 10 days before delivery) or recovered COVID-19 (green, PCR diagnosis >10 days before delivery). Triangles indicate tissues positive for SARS-CoV-2 viral RNA. Within each box plot, the horizontal line denotes the median and the top and bottom box borders reflect the 75th and 25th percentiles, respectively. *p<0.05, **p<0.01, ***p<0.001

**Figure 3.** Relationship Between Gestational Age at COVID-19 Diagnosis and Placental Innate Immune Gene Expression at Delivery in Pregnant Women with and without COVID-19 in Pregnancy. The x-axis indicates gestational age at COVID-19 diagnosis. Blue dots at week 0 indicate placental gene expression in uninfected pregnant women (controls) but are not included in linear fit displayed in this figure. The y-axis is relative gene expression to *TBP* (TATA-Box Binding Protein) for each innate immune gene studied. Red dots reflect cases with SARS-CoV-2 diagnosis 10 days prior to delivery (active COVID-19). Green dots indicate SARS-CoV-2 cases diagnosed >10 days before delivery (recovered COVID-19). Triangles indicate a tissue with detectable SARS-CoV-2 vRNA. Squares indicate placental tissues with SARS-CoV-2-associated placental pathology (Table 2). The line reflects the line of best fit and was non-significant in all cases.
Figure 4. Relationship Between COVID-19 Disease Severity and Placental Innate Immune Gene Expression at Delivery in Pregnant Women with and without COVID-19 in Pregnancy. Dot plots are shown to assess the potential correlation between COVID-19 disease severity (scored as asymptomatic, mild, moderate/severe) and the relative gene expression of IFNA2, IFNB, IFIT1, MXA, IL6, and IL1B to TBP (TATA-Box Binding Protein), a housekeeping gene, in placental tissues. Blue and black dots indicate placental tissues from subjects that labored and did not labor, respectively. Subjects with unknown disease severity are not shown. The blue and gray lines reflect the line of best fit for gene expression in the labored and unlabored subjects, respectively, and were non-significant in all cases.

Figure 5. Dot plots are shown to assess the potential correlation between the maternal pre-pregnancy body mass index (BMI) and relative gene expression of IFNA2, IFNB, IFIT1, MXA, IL6, and IL1B to TBP (TATA-Box Binding Protein), a housekeeping gene, in placental tissues from women with COVID-19 during pregnancy. The line of best fit is either gray to indicate the line of best fit for labored placentas or blue to indicate non-labored placentas. A significant positive correlation was identified between IFNB expression in non-labored CAM and pre-pregnancy maternal BMI such that a higher BMI correlated with greater IFNB expression.

Figure 6. Dot plots are shown to assess the potential correlation between placental pathology associated with SARS-CoV-2 infection (histiocytic chorionic villitis with/without perivillous fibrin deposition; Table S1) and the relative gene expression of IFNA2, IFNB,
IFIT1, MXA, IL6, and IL1B to TBP (TATA-Box Binding Protein), a housekeeping gene, in placental tissues from women with COVID-19 during pregnancy. The gray line of best fit connects either the black dots (labored placentas) with and without placental pathology; a blue line connects the blue dots (unlabored placentas) with and without placental pathology. A significant positive correlation was identified in the unlabored CAM tissues for IL6 and IL1B, indicating that SARS-CoV-2-associated placental pathology correlated with higher IL6 and IL1B gene expression.

Figure 7. Dot plots are shown to assess the potential correlation between time interval from COVID-19 diagnosis to delivery and relative gene expression of IFNA2, IFNB, IFIT1, MXA, IL6, and IL1B to TBP (TATA-Box Binding Protein), a housekeeping gene, in placental tissues. The line of best fit is either gray to indicate labored placentas or blue to indicate non-labored placentas. There was a significant positive correlation between IL1B gene expression (labored chorionic villous tissues) and COVID-19 infection duration, such that a longer interval from diagnosis was associated with higher Il1b gene expression. As preterm deliveries occurred in only 22/164 (13.4%) cases, this data also reflects correlations between gene expression and the gestational age at COVID-19 diagnosis. Note that earlier gestational ages at COVID-19 diagnosis are reflected in a longer time interval from infection to delivery.
The diagram shows a scatter plot with a logarithmic scale (Log2 Fold Change) on the y-axis and multiple groups on the x-axis, including IFNA2, IFNB, IFIT1, MXA, IL6, and IL1B. The groups are further categorized under Chorionic Membranes and Chorionic Villous Tissue. Each group contains data points represented by different symbols, and statistical significance is indicated by asterisks (**, ***).