Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

OBSTETRICS

Diminished antiviral innate immune gene expression in the placenta following a maternal SARS-CoV-2 infection

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BACKGROUND: COVID-19 is caused by the SARS-CoV-2 virus 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 the risk of inflammatory tissue injury or placental compromise and may 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 6 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 up-regulated placental antiviral innate immune response.

STUDY DESIGN: We performed a case—control study on placental tissues (chorionic villous tissues and chorioamniotic membrane) collected from pregnant patients with (N=140) and without (N=24) COVID-19 disease. We performed real-time quantitative polymerase chain reaction and immunohistochemistry, and the placental histopathology was evaluated. Clinical data were abstracted. Fisher exact test, Pearson correlations, and linear regression models were used to examine proportions and continuous data between patients with active (<10 days since diagnosis) vs recovered COVID-19 (>10 days since diagnosis) at the 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 viral RNA 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 viral RNA in placental tissues was confirmed to express SARS-CoV-2 nucleocapsid and spike proteins in syncytiotrophoblast cells. We detected a considerably lower gene expression of 5 critical innate immune mediators (*IFNB*, *IFIT1*, *MXA*, *IL6*, *IL1B*) in the chorionic villi and chorioamniotic membranes from women with active or recovered COVID-19 than 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 between COVID-19 diagnosis and delivery, prepregnancy 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, time interval from COVID-19 diagnosis to delivery, maternal obesity, disease severity, or placental pathology.

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

Introduction

Pregnant women who become infected with SARS-CoV-2 are more susceptible to hospitalization, critical illness, and numerous adverse perinatal complications (eg, stillbirth, preeclampsia, and preterm birth). They are also associated with a higher likelihood of requiring intensive care unit admission and mechanical ventilation and show 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 on 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 nonstructural protein 1 (Nsp1) and open reading frame 6 (ORF6) inhibit phosphorylation of signal transducer and activator-of-transcription (STAT)[25] proteins and impede messenger RNA (mRNA) production or processing while promoting host mRNA destruction.[25–27] Impaired STAT protein phosphorylation by

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AJOG at a Glance

Why was this study conducted?

This study aimed to evaluate the impact of maternal SARS-CoV-2 infection on placental innate immune response.

Key findings

We identified a marked reduction in gene expression of numerous innate immune mediators critical for antiviral host defense in the placental tissues in a large cohort of pregnant women diagnosed with COVID-19 during their pregnancy. The placental innate immune defenses were negatively impacted, regardless of COVID-19 disease severity, gestational age at COVID-19 diagnosis, and 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 of other infections in pregnancy or reflect a broader decline in placental function.

SARS-CoV-2 suppresses IFN expression and signaling, broadly evading innate immune responses.[27,28] To what extent a placental infection by SARS-CoV-2 modulates the placental antiviral innate immune response is unclear.[29−34] A robust or dysregulated innate immune response may increase the risk of inflammatory tissue injury or placental compromise and contribute to deleterious fetal outcomes, including stillbirth.[11,35−40] A spectrum of placental pathologic conditions 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.[9,19,41−48] Previous 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.

This study aimed 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 owing to smaller sample sizes (viz, labor status, placental pathology, time interval between infection and delivery, and COVID-19 disease severity). We hypothesized that placental antiviral response might be up-regulated 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 June 2020 and July 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 #00002410, informed consent). Coded placental samples and data from Intermountain Healthcare were sent to the University of Washington. As K.M.A.W. and her team did not have access to any personal identifiers linked to the Intermountain Healthcare samples, this study was deemed to not involve any 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] and chorionic villous [CV] tissues) 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 including prepregnancy body mass index (BMI, kg/m^2), 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 were abstracted from patient charts. Active COVID-19 and recovered COVID-19 were defined as delivery $\leq$ 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 the 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 or severe disease. COVID-19 disease

TABLE 1
Clinical characteristics and COVID-19 disease and pregnancy and neonatal outcomes

Characteristic/outcome	All (N=164)	Pregnant individuals		*P* value[a]
		Controls (N=24)	COVID-19 (N=140)	
Pregnancy and medical history				
Parity	2.6±1.6	2.5±1.3	2.6±1.6	.793
Prepregnancy BMI (kg/m^2)	28.6±6.6	27.6±6.7	28.7±6.6	.466
Obesity (BMI ≥30.0 kg/m^2)	58 (35.4%)	6 (25.0%)	52 (37.1%)	.356
Asthma	3 (1.8%)	0 (0%)	3 (2.1%)	1.000
Diabetes mellitus	2 (1.2%)	1 (0.6%)	1 (0.6%)	1.000
Chronic hypertension	8 (4.9%)	3 (12.5%)	5 (3.6%)	.094
Pregnancy and neonatal outcomes				
Gestational age at delivery (wk, N=162)	37.9±2.2	38.5±1.2	37.8±2.4	.050
Preterm birth rate	22 (13.4%)	1 (4.2%)	21 (15.0%)	.203
PPROM	8 (4.9%)	0 (0%)	8 (5.7%)	.605
Preeclampsia and gestational hypertension	19 (11.6%)	2 (8.3%)	17 (12.1%)	.743
Mode of delivery				.107
Cesarean delivery	57 (34.8%)	12 (50.0%)	45 (32.1%)	
Vaginal delivery	107 (65.2%)	12 (50.0%)	95 (67.9%)	
Labor				.004
Yes	124 (75.6%)	12 (50.0%)	112 (80.0%)	
No	40 (24.4%)	12 (50.0%)	28 (20.0%)	
Fetal birthweight (g)	3156.6±603.5	3421.0±496.3	3,111.3±610	.010
Apgar score at 1 min (N=154; 20 control, 134 COVID-19)	7.67±1.15	7.90±0.31	7.63±1.22	.037
Apgar score at 5 min (N=154; 20 control, 134 COVID-19)	8.75±0.85	8.70±0.57	8.76±0.89	.683
COVID-19 disease (N=140)				
Gestational age at COVID-19 diagnosis	—	—	30.6±8.1	—
COVID-19 diagnosis to delivery (wk; N=138)	—	—	7.2±8.0	—
COVID-19 status at delivery				—
Active COVID-19	—	—	51 (36.4%)	
Recovered COVID-19	—	—	89 (63.6%)	
COVID-19 symptoms/severity			34 (24.3%)	—
None	—	—	71 (50.7%)	
Mild	—	—	14 (10.0%)	
Moderate or severe	—	—	21 (15.0%)	
Unknown	—	—		

Information about maternal age and fetal sex was not available for most of the cohort and is therefore not reported.

BMI, body mass index; *NS*, nonsignificant; *PPROM*, preterm premature rupture of membranes.

[a] *P* values are considered significant if <.05.

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severity was defined as asymptomatic disease (no reported symptoms), mild (pneumonia symptoms of fever, cough, sore throat, and muscle pain without shortness of breath), moderate (dyspnea, respiratory rate ≥30 breaths per minute, percutaneous oxygen saturation ≤93% on room air at rest, arterial oxygen tension over inspiratory oxygen

TABLE 2
Cases and controls with SARS-CoV-2-associated placental pathology

Case	Group	Placental pathology notes	SARS-CoV-2 viral RNA detected
1	COVID-19	Borderline increase in perivillous fibrin deposition without histiocytes encasing anchoring villi and adjacent villi	No
2	COVID-19	Multiple small foci of perivillous fibrin deposition with histiocytic intervillous inflammation, 1 focus with active villous inflammation	No
3	COVID-19	Multiple small foci of perivillous fibrin deposition with histiocytic intervillous inflammation	No
4	COVID-19	Small group of villi with chronic villitis	No
5	COVID-19	Multiple foci of perivillous fibrin deposition and probable villitis without histiocytic intervillous inflammation	Yes
6	COVID-19	Single tiny focus of perivillous fibrin deposition with histiocytic intervillous inflammation	No
7	COVID-19	Fetal side of disc with single focus of perivillous fibrin deposition and histiocytic intervillous inflammation	No
8	COVID-19	Single tiny focus of chronic villitis	No
9	COVID-19	Focal chronic villitis	No
10	COVID-19	Diffuse perivillous fibrin deposition with histiocytic intervillous inflammation.	Yes
11	COVID-19	Basal zone of perivillous fibrin deposition with histiocytic intervillous inflammation	No
12	COVID-19	Widespread placental basal infarcts, trophoblast necrosis, chronic villitis, and diffuse perivillous fibrin deposition with histiocytic intervillous inflammation	No
13	COVID-19	Single minute focus of chronic villitis	No
14	COVID-19	Borderline increase in perivillous fibrin deposition without histiocytes encasing anchoring villi and adjacent villi	No
15	COVID-19	Basal zone lymphohistiocytic villitis and fibrin deposition just along the decidual interface	No
16	Control	Patchy perivillous fibrin deposition	No
17	Control	Patchy perivillous fibrin deposition; 1 focus of chronic villitis	No
18	Control	2 tiny foci of chronic villitis	No

This table reflects notes from the placental pathologist (R.P.K.) for each case that was considered to have chronic villitis or 1 or more features 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.

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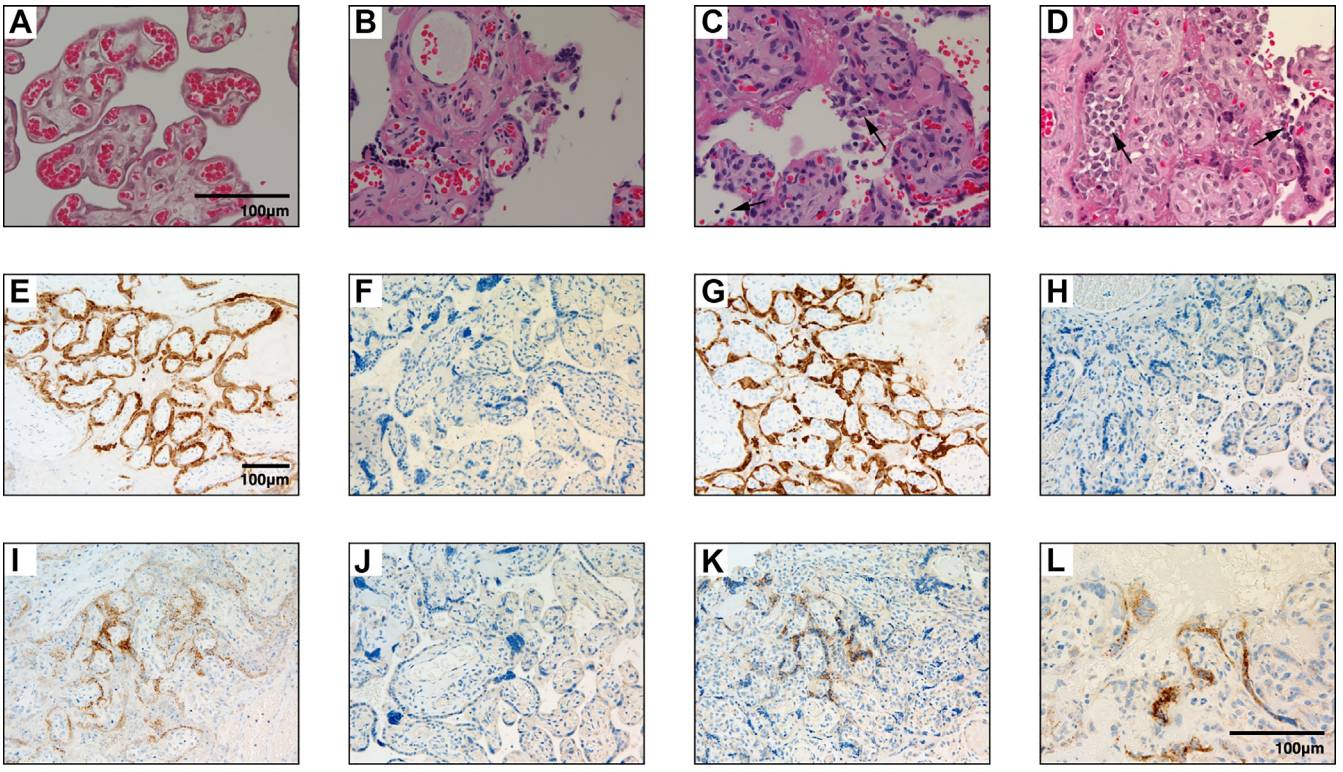
fraction of <300 mm Hg, and/or lung infiltrates >50% within 24–48 hours), and severe (severe respiratory distress, respiratory failure requiring mechanical ventilation, shock, and/or multiple organ dysfunction or failure). All laboratory assays, histopathology analysis, and statistical analysis were performed at the University of Washington. Coded 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, the 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. The RNA concentrations were measured by nanodrop, which reported A260/A280 ratios that confirmed acceptable purity without considerable DNA or protein contamination. Next, reverse transcription and complementary DNA 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

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 3 pregnant individuals with a history of COVID-19 during the pregnancy (**B**—**D**). Placental pathology included perivillous fibrin deposition and intervillous 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 Figure (**E**) and (**I**) from a SARS-CoV-2 polymerase chain reaction-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 (**H** for nucleocapsid) and SARS-CoV-2 immunostaining of tissues from a healthy, uninfected control (**F** and **J**). SARS-CoV-2 antigen staining was demonstrated in the placental syncytiotrophoblast of 1 of 5 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 (**G**, **K**, **L**) was identical to 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**.

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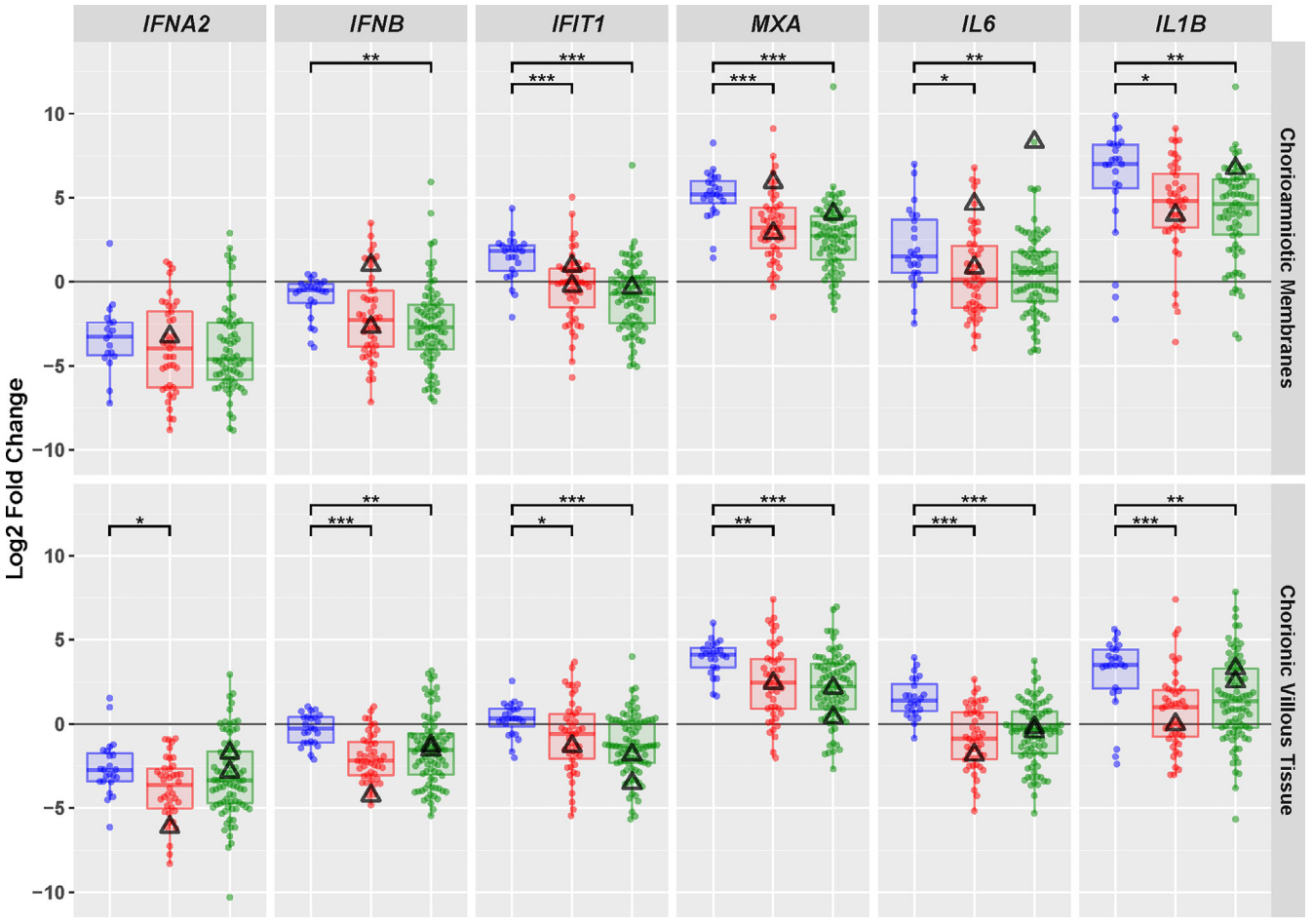
binding protein). Reaction mixtures were made using the Taqman Fast Advance Master Mix (cat# 4444964; Thermo Fisher Scientific Inc, Waltham, MA), SARS-CoV-2 N2 primers and probes from the 2019-nCoV RUO kit (10006713; Integrated DNA Technologies MNC, XX) with an added quencher, TBP primers and probes, and nuclease-free water. Viral load standards, positive control, no treatment control, and 400 ng 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 (Thermo Fisher Scientific Inc). 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/CCC CAGCGCTTCAG-3IABkF-3'. The N2 forward primer sequence was 5'-TTA-CAAACATTGGCCGCAAA-3'. The N2 reverse primer sequence was 5'-GCG CGACATTCCGAAGAA-3'. The TBP probe sequence was: 5'- Quasar670-CA CAGGAGCCAAGAGTGAAGAACAGT-BHQ-2-3'. The TBP Primer/Probe was obtained from Thermo Fisher (catalog# Hs00427620_m1; Thermo Fisher Scientific, Inc). *TBP* amplification was performed in duplex with SARS-CoV-2 N2 for 2 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;

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 with expression of the TATA Box Binding Protein (*TBP*, housekeeping gene). Gene expression is shown in uninfected control pregnant women (*blue*) compared with 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. *Single asterisk* represents $P<.05$, *double asterisks* represent $P<.01$, *triple asterisks* represent $P<.001$.

IFN, interferon; *IL*, interleukin; *PCR*, polymerase chain reaction.

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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 (Thermo Fisher Scientific 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 (R.P.K.), who was blinded to the patient's COVID-19 status. We focused analysis of placental pathology on the following key features linked to SARS-CoV-2 infection: chronic histiocytic intervillositis, perivillous fibrin deposition, and trophoblast necrosis. Villitis and other forms of CV 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

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

	CAM (CTL: N=18; ACT: N=41; RECOV: N=68)							CV (CTL: N=23; ACT: N=40; RECOV: N=83)						
	Model 1				Model 2				Model 1				Model 2			
Predictors	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*
CTL (Intercept)	−3.31	0.60	−5.49	<.001[a]	−2.62	0.64	−4.08	<.001[a]	−2.58	0.44	−5.84	<.001[a]	−2.30	0.49	−4.69	<.001[a]
ACT	−0.60	0.72	−0.83	.407	−0.25	0.72	−0.34	.731	−1.28	0.55	−2.31	.022[a]	−1.13	0.56	−2.01	.047[a]
RECOV	−0.72	0.68	−1.06	.291	−0.33	0.68	−0.49	.624	−0.51	0.50	−1.02	0.311	−0.36	0.51	−0.71	.477
Labored (Yes)					−1.38	0.51	−2.70	.008[a]					−0.52	0.42	−1.26	.211
Observations	127				127				146				146			
R^2/R^2 adjusted	0.009/−0.007				0.065/0.042				0.041/0.027				0.051/0.031			

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. 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.

ACT, active COVID-19; *CAM*, chorioamniotic membranes; *Coeff*, coefficient; *CTL*, controls without a history of COVID-19; *CV*, chorionic villus tissue; *RECOV*, recovered COVID-19; *SE*, standard error.

[a] Significant *P* values.

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sections from formalin-fixed placental samples using a Ventana Benchmark II automated immunostainer and 2 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 the "R" software (R Core Team, 2022).[51] To examine relationships between discrete variables, we used the Fisher exact test, and for relationships between continuous variables, we used Pearson correlations. 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 2 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 nonlabored) was added as a covariate. A *P* value <.05 was considered statistically significant. The data sets

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

	CAM (CTL: N=18; ACT: N=41; RECOV: N=68)							CV (CTL: N=23; ACT: N=40; RECOV: N=83)						
	Model 1				Model 2			Model 1				Model 2		
Predictors	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*
CTL (Intercept)	−0.93	0.48	−1.95	.053	−0.36	0.52	−0.69	.489	−0.31	0.36	−0.88	.381	−0.14	0.39	−0.36	.716
ACT	−1.11	0.58	−1.91	.058	−0.78	0.59	−1.33	.186	−1.69	0.44	−3.83	<.001[a]	−1.59	0.45	−3.51	.001[a]
RECOV	−1.66	0.55	−3.04	.003[a]	−1.33	0.55	−2.42	.017[a]	−1.24	0.41	−3.07	.003[a]	−1.15	0.42	−2.76	.007[a]
Labored (Yes)					−1.14	0.44	−2.57	.011[a]					−0.34	0.33	−1.01	.313
Observations	147				147				152				152			
R^2/R^2 adjusted	0.061/0.048				0.103/0.084				0.091/0.079				0.097/0.079			

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. 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.

ACT, active COVID-19; *CAM*, chorioamniotic membranes; CTL, controls without a history of COVID-19; *CV*, chorionic villus tissue; *RECOV*, recovered COVID-19; *Coeff*, coefficient; *SE*, standard error.

[a] Significant *P* values.

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generated 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. The clinical characteristics and 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, were multiparous, and were overweight before pregnancy (Table 1). The mean gestational age at delivery was similar between pregnant women with COVID-19 and healthy controls (COVID-19: 37.8±2.4 vs controls: 38.5±1.2; t=−2.00; *P*=.05). A greater proportion of individuals with COVID-19 labored before delivery than healthy controls (80.0% vs 50.0%, *P*=.004). Preterm birth (<37 weeks) occurred in 21 of 140 pregnant women with COVID-19 (15.0%; *P*=.2) and in 1 of 24 controls (4.2%; *P*=.203). Fetal birthweight was significantly lower in the COVID-19 group (3111.3±610.0 vs 3421.0±496.3; t=−2.73; *P*=.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*=.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 weeks, 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 or 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. We next quantified SARS-CoV-2 viral load in placental tissues to determine whether

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

	CAM (CTL: N=18; ACT: N=41; RECOV: N=68)							CV (CTL: N=23; ACT: N=40; RECOV: N=83)						
	Model 1				Model 2			Model 1				Model 2		
Predictors	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*
CTL (Intercept)	1.43	0.40	3.60	<.001[a]	2.10	0.42	4.97	<.001[a]	0.29	0.38	0.77	.441	0.60	0.41	1.44	.151
ACT	−1.72	0.48	−3.55	.001[a]	−1.31	0.48	−2.75	.007[a]	−1.03	0.46	−2.21	.029[a]	−0.84	0.47	−1.77	.078
RECOV	−2.33	0.45	−5.12	<.001[a]	−1.91	0.45	−4.25	<.001[a]	−1.58	0.43	−3.69	<.001[a]	−1.40	0.44	−3.20	.002[a]
Labored (Yes)					−1.35	0.37	−3.68	<.001[a]					−0.61	0.35	−1.73	.085
Observations	152				152				152				152			
R^2/R^2 adjusted	0.150/0.139				0.221/0.206				0.086/0.073				0.104/0.086			

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. 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.

CAM, chorioamniotic membranes; CTL, controls without a history of COVID-19; *CV*, chorionic villus tissue; ACT, active COVID-19; RECOV, recovered COVID-19; Coeff., coefficient; SE, standard error.

[a] Significant *P* values.

Color. Placental innate immune suppression after maternal COVID-19. Am J Obstet Gynecol 2022.

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 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 and from 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 (Figure 1, E–G) and spike (Figure 1, I–L) proteins in syncytiotrophoblast cells. In this case of active COVID-19, the viral load in CV tissues was high (1.2×10^{11} copies/mg) and was associated with widespread placental basal infarcts, trophoblast necrosis, chronic villitis, and diffuse perivillous fibrin deposition with histiocytic intervillous inflammation (Figure 1, B–1D). Of significance, this patient experienced preterm premature rupture of membranes and delivered a preterm infant at 33 weeks gestation 6 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=.09).

Next, we evaluated the 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 (Figure 2). These genes represent key modulators of antiviral innate immunity: IFN-β, IL1β, and IFN-α2 coordinate inflammatory and antiviral

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

	CAM (CTL: N=18; ACT: N=41; RECOV: N=68)							CV (CTL: N=23; ACT: N=40; RECOV: N=83)						
	Model 1				Model 2			Model 1				Model 2		
Predictors	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*
CTL (Intercept)	5.14	0.40	12.86	<.001[a]	5.83	0.42	13.74	<.001[a]	3.94	0.40	9.89	<.001[a]	4.16	0.44	9.47	<.001[a]
ACT	−1.87	0.49	−3.85	<.001[a]	−1.46	0.48	−3.05	.003[a]	−1.41	0.49	−2.87	.005[a]	−1.27	0.50	−2.53	.012[a]
RECOV	−2.49	0.46	−5.47	<.001[a]	−2.08	0.45	−4.61	<.001[a]	−1.78	0.45	−3.94	<.001[a]	−1.65	0.46	−3.57	<.001[a]
Labored (Yes)					−1.37	0.36	−3.79	<.001[a]					−0.44	0.37	−1.18	.240
Observations	155				155				156				156			
R^2/R^2 adjusted	0.165/0.154				0.237/0.222				0.092/0.080				0.101/0.083			

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. 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.

ACT, active COVID-19; *CAM*, chorioamniotic membranes; *Coeff*, coefficient; *CTL*, controls without a history of COVID-19; *RECOV*, recovered COVID-19; *SE*, standard error.

Coler. Placental innate immune suppression after maternal COVID-19. Am J Obstet Gynecol 2022.

[a] Significant *P* values.

immune actions, MxA and IFIT1 inhibit viral replication, and IL-6 coordinates broad inflammatory and immune responses.[52,53] Pregnant women with recovered COVID-19 in CAM (Figure 2) and CV (Figure 2) tissues had significantly diminished expression of *IFNB, IFIT1, MXA, IL6,* and *IL1B* (all, $P<.05$). 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 with the housekeeping gene (8.3-fold) (Figure 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, $P<.05$). In contrast, gene expression of *IFNA2* was similar between the healthy controls and COVID-19 groups except for significantly diminished gene expression in the CV active COVID-19 group ($P<.05$). 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 ($P<.05$). In each case, there was evidence for significant stochastic ordering (P values $<.01$), providing further support for altered placental innate immune gene expression in pregnant women with COVID-19. In summary, both 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. On adjusting for labor status, multiple linear regression analyses determined that the patterns of gene expression across controls and

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

	CAM (CTL: N=18; ACT: N=41; RECOV: N=68)							CV (CTL: N=23; ACT: N=40; RECOV: N=83)						
	Model 1				Model 2			Model 1				Model 2		
Predictors	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*
CTL (Intercept)	2.00	0.50	3.99	<.001[a]	1.16	0.54	2.17	.032[a]	1.53	0.36	4.30	<.001[a]	1.67	0.39	4.25	<.001[a]
ACT	−1.45	0.61	−2.38	.019[a]	−1.96	0.60	−3.25	.001[a]	−2.34	0.44	−5.34	<.001[a]	−2.25	0.45	−5.00	<.001[a]
RECOV	−1.57	0.57	−2.74	.007[a]	−2.09	0.57	−3.67	<.001[a]	−2.08	0.40	−5.15	<.001[a]	−2.00	0.42	−4.81	<.001[a]
Labored (Yes)					1.69	0.46	3.65	<.001[a]					−0.28	0.33	−0.85	.399
Observations	152				152				156				156			
R^2/R^2 adjusted	0.050/0.037				0.128/0.111				0.174/0.163				0.177/0.161			

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. 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.

ACT, active COVID-19; *CAM*, chorioamniotic membranes; *Coeff*, coefficient; *CTL*, controls without a history of COVID-19; *RECOV*, recovered COVID-19; *SE*, standard error.

[a] Significant *P* values.

Color. Placental innate immune suppression after maternal COVID-19. Am J Obstet Gynecol 2022.

COVID-19 groups did not change substantially in either CAM or CV tissues (Tables 3−8). Across 24 comparisons involving 2 tissues (CV and CAM), 2 groups (active and recovered), and 6 genes (*IFNA2, IFNB, IFIT1, MXA*, *IL6,* and *IL1B*), there were 20 significant differences between either active or recovered COVID-19 and the corresponding control group ($P<.05$). After adjustment for labor, 18 significant differences ($P<.05$) remained with the loss of *IFIT1* (active COVID-19 vs controls in CV; $P=.08$) (Table 5) and *IL1B* (active COVID-19 vs controls in CAM; $P=.08$) (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 (Figure 3), COVID-19 disease severity (Figure 4), maternal prepregnancy BMI (Figure 5), time interval between COVID-19 diagnosis and delivery (Figure 6), and detection of SARS-CoV-2-associated placental pathology (Figure 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 ($P=.04$) (Figure 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*: $P=.004$, *IL1B*: $P=.02$) (Figure 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

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

	CAM (CTL: N=18; ACT: N=41; RECOV: N=68)							CV (CTL: N=23; ACT: N=40; RECOV: N=83)						
	Model 1				Model 2				Model 1				Model 2			
Predictors	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*	Est	SE	Stat	*P*
CTL (Intercept)	6.04	0.59	10.25	<.001[a]	6.38	0.65	9.87	<.001[a]	3.01	0.49	6.10	<.001[a]	2.95	0.55	5.39	<.001[a]
ACT	−1.52	0.73	−2.08	.039[a]	−1.32	0.75	−1.76	.080	−2.23	0.61	−3.63	<.001[a]	−2.26	0.63	−3.59	<.001[a]
RECOV	−1.84	0.67	−2.74	.007[a]	−1.61	0.70	−2.32	.022[a]	−1.59	0.56	−2.83	.005[a]	−1.62	0.58	−2.80	.006[a]
Labored (Yes)					−0.71	0.56	−1.27	0.206					0.12	0.47	0.26	0.798
Observations	142				142				152				152			
R^2/R^2 adjusted	0.052/0.038				0.063/0.042				0.082/0.070				0.083/0.064			

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. 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.

ACT, active COVID-19; *CAM*, chorioamniotic membranes; *Coeff*, coefficient; *CTL*, controls without a history of COVID-19; *RECOV*, recovered COVID-19; *SE*, standard error

[a] Significant *P* values.

Color. Placental innate immune suppression after maternal COVID-19. Am J Obstet Gynecol 2022.

expression, placental pathology, and time interval between infection and delivery ($P<.05$).

Comment

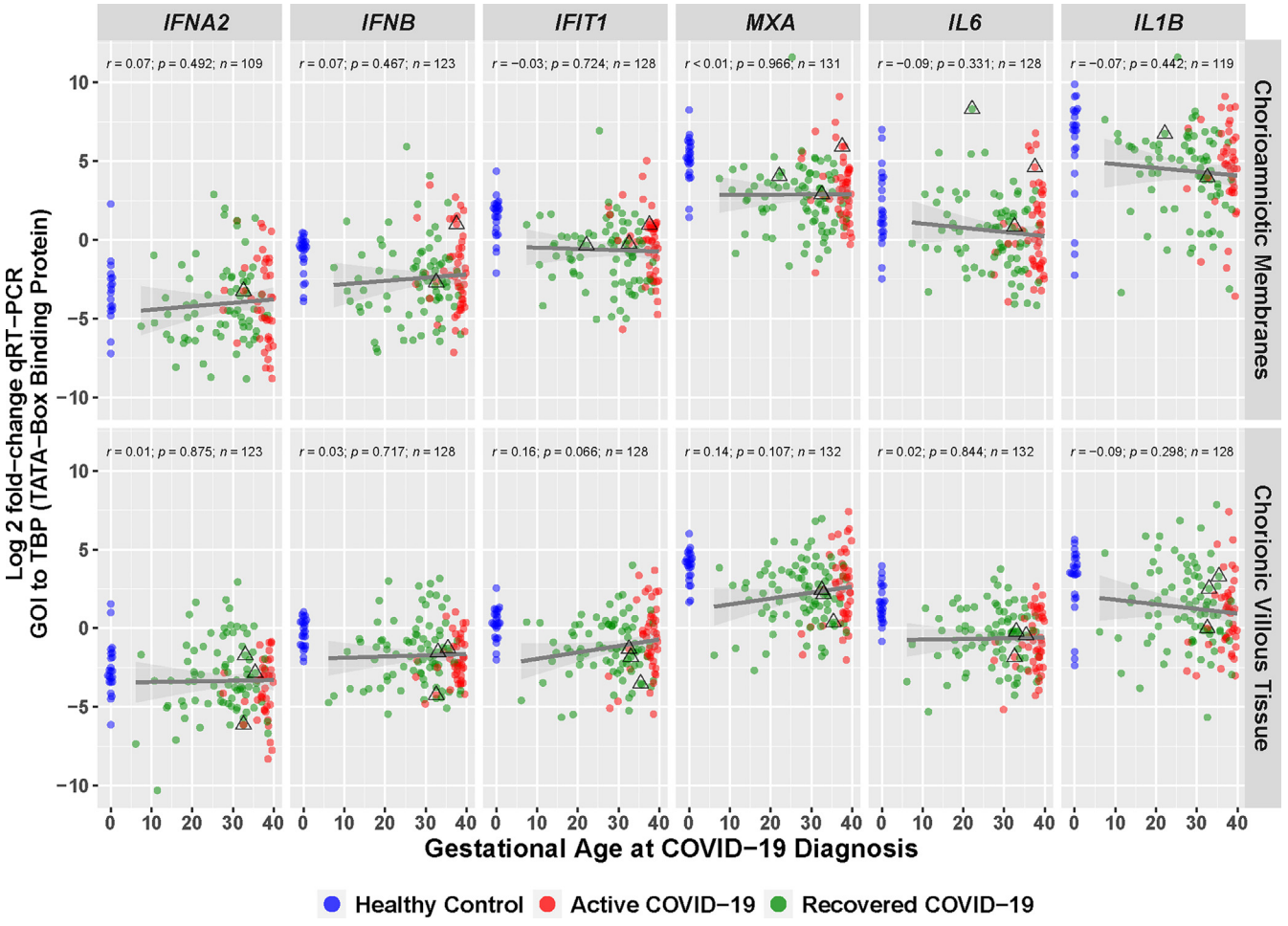
Principal findings

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 (Video 1). 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 ($P<.05$) after controlling for labor status, a known confounder owing to inflammatory processes occurring during labor and delivery.[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 between COVID-19 diagnosis and delivery, prepregnancy BMI, 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.

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 the risk of subsequent infection. In murine models of murine gammaherpesvirus-

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 before 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 nonsignificant in all cases.

IFN, interferon; *IL*, interleukin.

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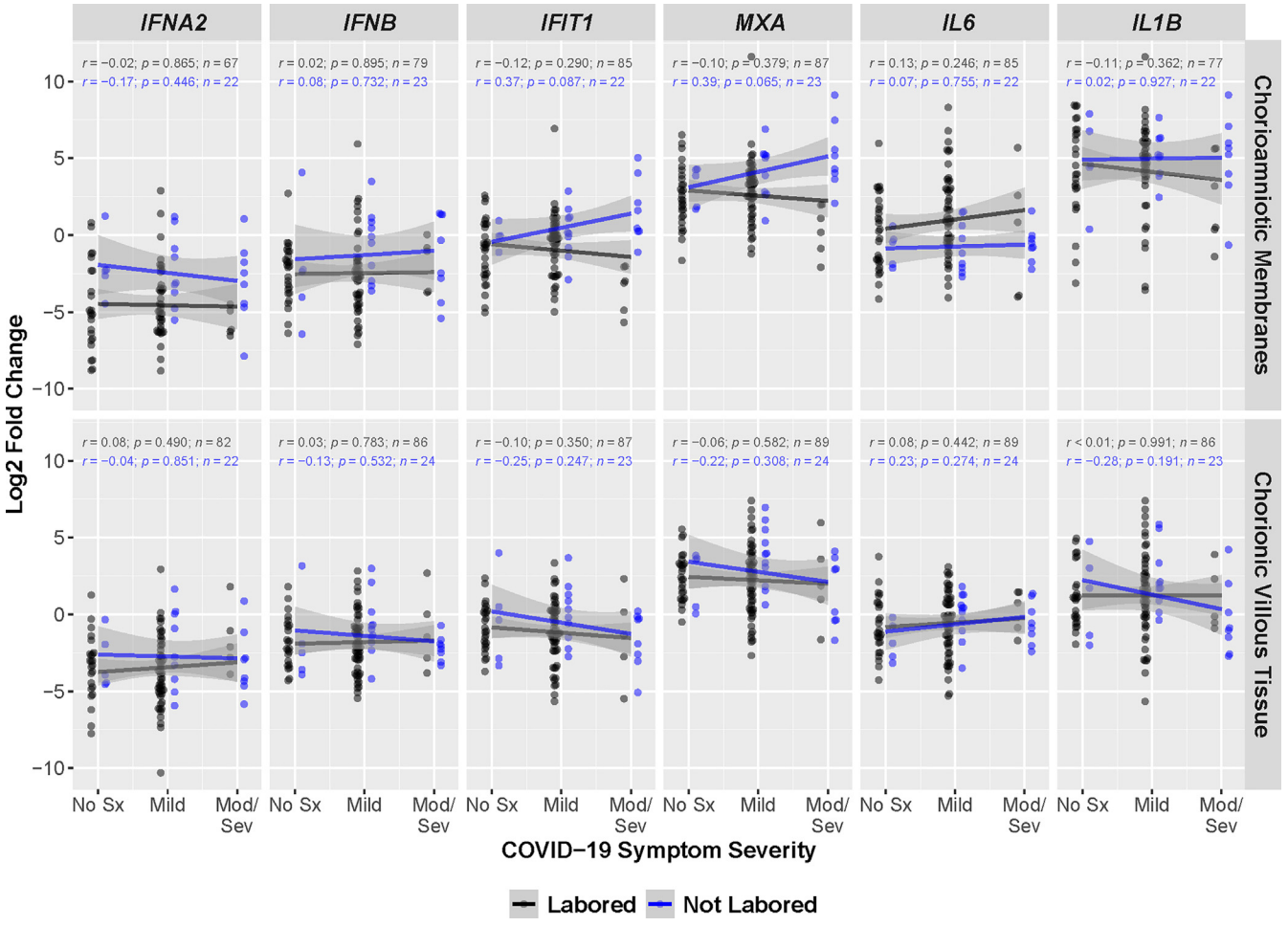
68 (MHV-68) infection, a subclinical viral infection sensitized maternal immune responses to coinfection or subsequent infection by other microorganisms.[56,57] Viral infections in these models compromised innate immune responses in cervical tissues, predisposing patients to intrauterine bacterial infections. MHV-68 diminished inflammatory and immune responses through decreased toll-like receptor gene expression and subsequent down-regulation of cytokine and chemokine gene expression that altered proinflammatory responses against bacterial pathogens.[56,57]

Research implications

Our results present several important research questions and generate new hypotheses. First, we hypothesize that impaired innate immunity is only 1 aspect of placental function that is impaired following a SARS-CoV-2 infection, likely owing 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.[40,58,59] As the canonical receptors for SARS-CoV-2 are not typically coexpressed in the placenta and viral

FIGURE 4

Relationship between COVID-19 symptom 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, and moderate or 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 nonsignificant in all cases.

IFN, interferon; *IL*, interleukin.

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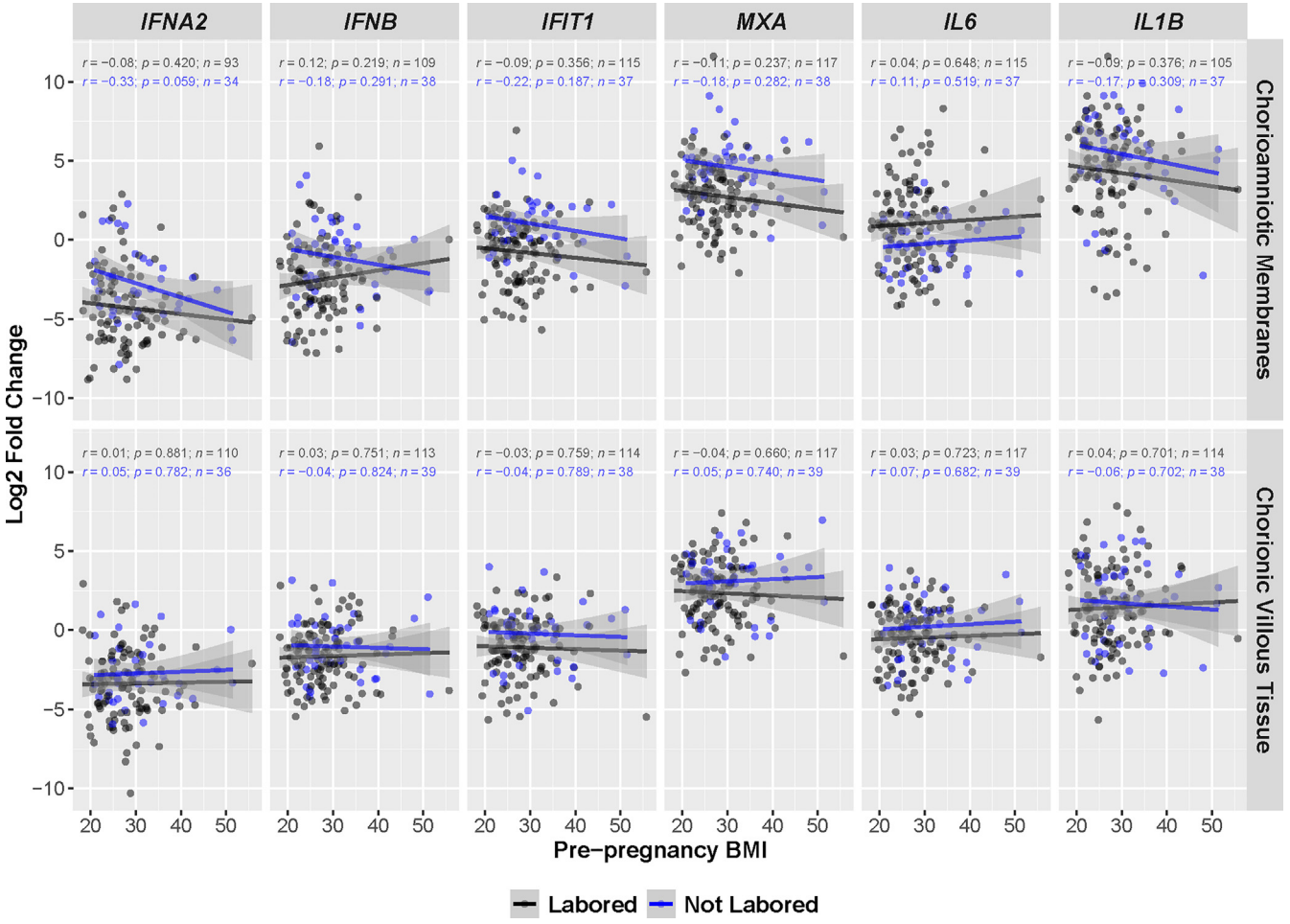
infection of syncytiotrophoblast cells appears rare, it is more probable that innate immune mediators released during an acute infection are the source of placental injury than a primary viral infection itself.[20,60] Regardless of the mechanism of placental injury, an impairment of placental immune functioning is likely to parallel other defects in metabolic and biological pathways that should be defined 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.[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 and also 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.[16] In pregnant individuals with mild COVID-19, there was also evidence for enrichment of decidual IL-1β-producing macrophages and an attenuation of interferon signaling in the decidua.[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

FIGURE 5
Correlation between maternal BMI and placental innate immune gene expression



Dot plots are shown to assess the potential correlation between the maternal prepregnancy 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 nonlabored placentas.

BMI, body mass index; *IFN*, interferon; *IL*, interleukin.

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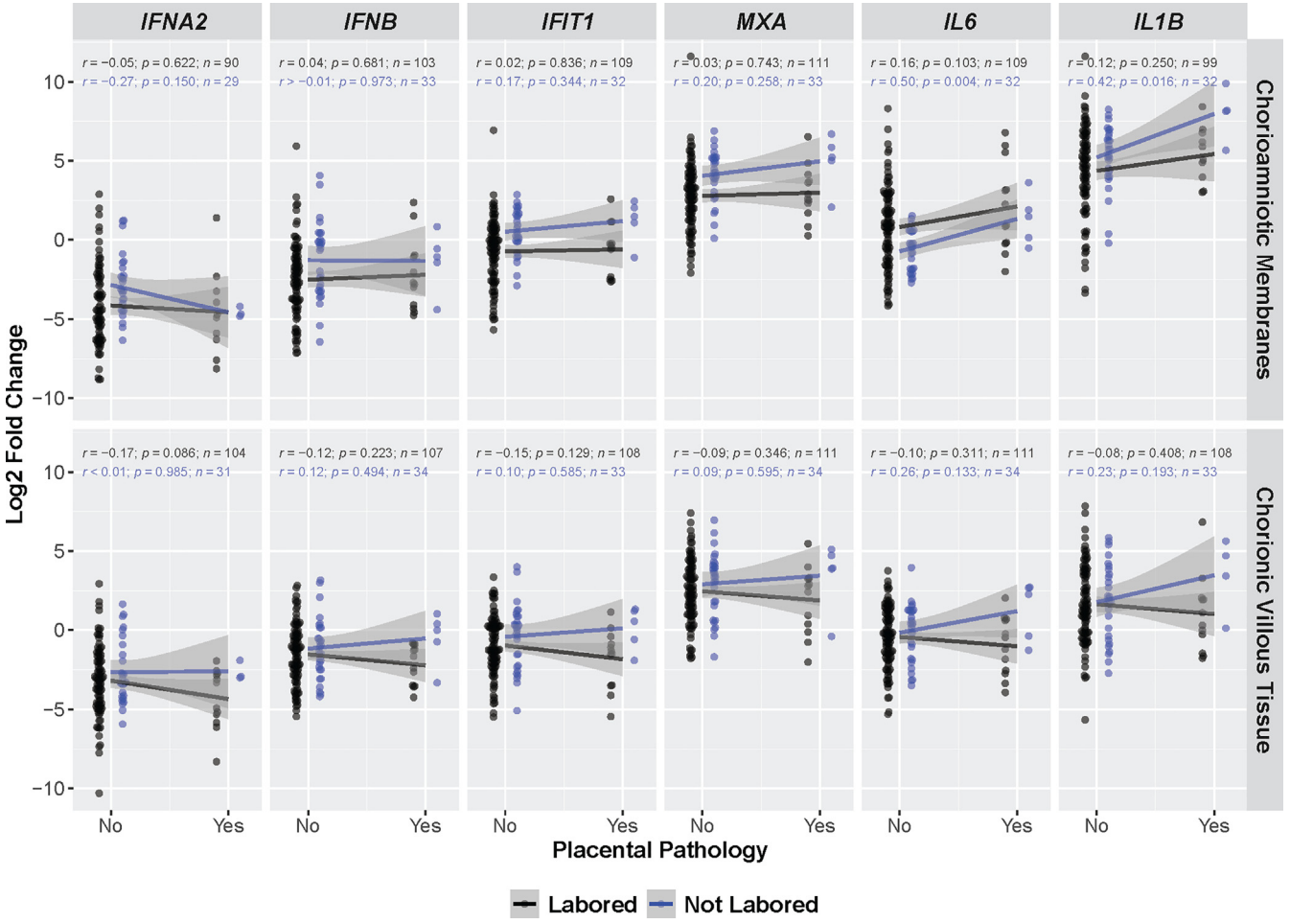
decidual subsets whereas innate immunity becomes quickly attenuated in the CV and CAM following infection. Finally, the temporal course of the SARS-CoV-2 innate immune response in maternal decidua and human placentas is not well-defined, as most samples have been collected at birth days to weeks and 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 because of 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.[64] 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

FIGURE 6
Correlation between placental pathology and placental innate immune gene expression



Dot plots are shown to assess the potential correlation between placental pathology associated with SARS-CoV-2 infection (histiocytic chorionic villitis with or 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 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 chorioamniotic membranes tissues for *IL6* (P=.004) and *IL1B* (P=.016), indicating that SARS-CoV-2-associated placental pathology correlated with higher *IL6* and *IL1B* gene expression.

IFN, interferon; *IL*, interleukin.

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not be concordant in their expression of viral RNA and protein.

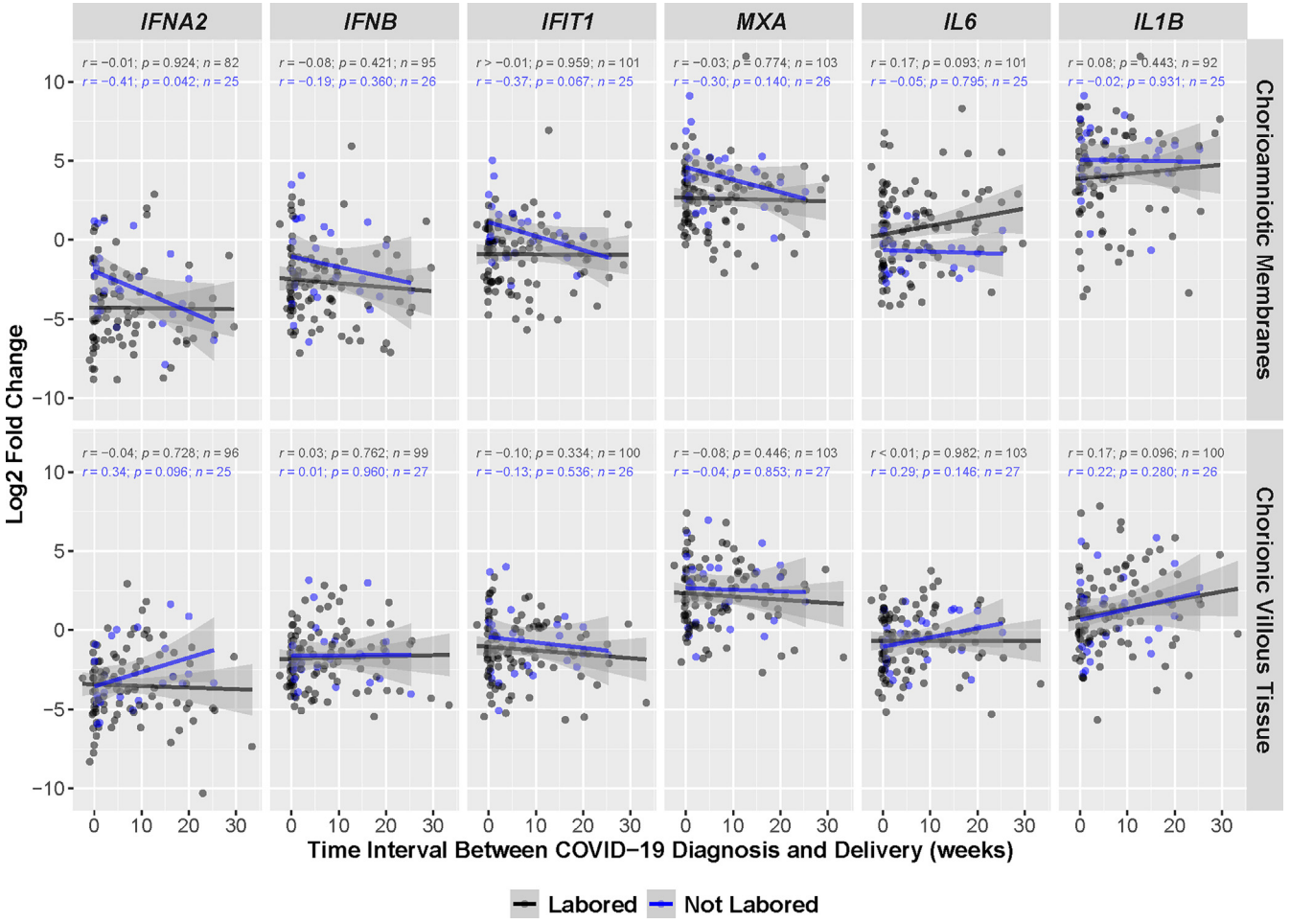
Clinical implications

These data have 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 with historic published studies from unexposed placentas (4%–20%).[45] Large studies evaluating rates of chorioamnionitis after a natural COVID-19 infection are needed to determine if the 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,[4,25,37,41,65–69] the impact on 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

FIGURE 7

Correlation between time interval from COVID-19 diagnosis to delivery and placental innate immune gene expression



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 nonlabored placentas. There was a significant positive correlation between *IFNA2* gene expression (labored chorionic villous tissues) and COVID-19 infection duration such that a longer interval from diagnosis was associated with higher *IFNA2* gene expression (P=.04). As preterm deliveries occurred in only 22/164 (13.4%) cases, these data also reflect 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.

IFN, interferon; *IL*, interleukin.

Coler. Placental innate immune suppression after maternal COVID-19. Am J Obstet Gynecol 2022.

adversely impact fetal neurodevelopment and increase the risk of neuropsychiatric and developmental disorders.[70] A large body of literature supports a link between diverse maternal infections and abnormal fetal neurodevelopment.[39,71–81] 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).[82] 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.[71]

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 were greater odds of having a neurodevelopmental diagnosis in infants exposed to a maternal SARS-CoV-2 infection (14/222; 6.3%) vs a healthy pregnant control group (227/7550; 3.0%) in the first 12 months of life. This study was limited by cohort size, 1 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 than children from uninfected pregnancies. Based on our previous 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 the rates of autism spectrum disorder, and 25 to 30 years of follow-up will be needed to evaluate differences in the rates of psychosis and schizophrenia.[39]

We recognize the enormity of the public health challenge to survey the neurodevelopment of all children exposed to SARS-CoV-2 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 United States 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 to 2016 may provide a useful toolkit for investigators to follow the neurodevelopment of children exposed to SARS-CoV-2 in utero.[83–86] Importantly, the adverse impact of fetal exposure to infectious diseases can impact neurodevelopment and mental health through adolescence and into adulthood.[39,71] 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.

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.[17,32,33,42–44,47,48,87–89] Our study is one of the earliest 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.[90,91] 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.

The 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 up-regulate the innate immune response before 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 1 subject delivered twins; our clinical data do not reveal whether 1 or 2 placentas were sampled from this unique patient.

Conclusions

We determined that a maternal SARS-CoV-2 infection can substantially 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 time point 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 the risks of stillbirth, chorioamnionitis, and other adverse health outcomes for the child. ■

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