The clinical impact of maternal COVID-19 on mothers, their infants, and placentas with an analysis of vertical transfer of maternal SARS-CoV-2-specific IgG antibodies

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ABSTRACT

Introduction: The effect of SARS-CoV-2 severity or the trimester of infection in pregnant mothers, placentas, and infants is not fully understood.
Methods: A retrospective, observational cohort study in Chapel Hill, NC of 115 mothers with SARS-CoV-2 and singleton pregnancies from December 1, 2019 to May 31, 2021 via chart review to document the infants' weight, length, head circumference, survival, congenital abnormalities, hearing loss, maternal complications, and placental pathology classified by the Amsterdam criteria.
Results: Of the 115 mothers, 85.2% were asymptomatic (n = 37) or had mild (n = 61) symptoms, 13.0% had moderate (n = 9) or severe (n = 6) COVID-19, and 1.74% (n = 2) did not have symptoms recorded. Moderate and severe maternal infections were associated with increased C-section, premature delivery, infant NICU admission, and were more likely to occur in Type 1 (p = 0.0055) and Type 2 (p = 0.0285) diabetic mothers. Only one infant (0.870%) became infected with SARS-CoV-2, which was not via the placenta. Most placentas (n = 63, 54.8%) did not show specific histologic findings; however, a subset showed mild maternal vascular malperfusion (n = 26, 22.6%) and/or mild microscopic ascending intrauterine infection (n = 28, 24.3%). The infants had no identifiable congenital abnormalities, and all infants and mothers survived.
Discussion: Most mothers and their infants had a routine clinical course; however, moderate and severe COVID-19 maternal infections were associated with pregnancy complications and premature delivery. Mothers with pre-existing, non-gestational diabetes were at greatest risk of developing moderate or severe COVID-19. The placental injury patterns of maternal vascular malperfusion and/or microscopic ascending intrauterine infection were not associated with maternal COVID-19 severity.

1. Introduction

SARS-CoV-2 is a novel enveloped single positive stranded RNA virus of the coronavirus family [1] that has infected hundreds of millions of individuals resulting in over 6 million deaths worldwide. This study was initiated at the beginning of the SARS-CoV-2 pandemic when little was known about the effects in pregnancy. The primary goal of this study was to determine the effect of the maternal severity and trimester of SARS-CoV-2 infection on mothers, placentas, and infants. A secondary goal was to determine the incidence of SARS-CoV-2 transmission to infants and the survival outcomes of the mothers and their infants.

Since the initiation of our study, there have been increasing reports on the effects of SARS-CoV-2 on the placenta which have varied with respect to their histologic findings of patterns of placental injury as

classified by the Amsterdam consensus statement [2]. A small and early study [3] reported increased fetal vascular malperfusion (FVM) in SARS-CoV-2-positive mothers. Two later studies [4,5] also found increased FVM compared to placentas from SARS-CoV-2 negative mothers. The FVM was mild or low grade in these studies. Conversely, other studies [6,7] have reported increased maternal vascular perfusion (MVM) compared to control placentas, with no increase in the other patterns of placental injury. A structured review of the literature [8], found a mean of 35% of the placentas from SARS-CoV-2-positive mothers showed FVM and 46% showed MVM. A recent systematic review and meta-analysis of 1008 placentas after SARS-CoV-2 maternal infection [9] reported MVM in 30.7%, FVM in 27.08%, acute inflammatory pathology in 22.68%, and chronic inflammatory pathology in 25.65%. Finally, other studies [10–13] showed no association with a specific placental pathology in placentas from SARS-CoV-2 mothers.

All studies to date have shown the incidence of *trans*-placental viral transmission to be very rare and when there is SARS-CoV-2 infection of the placenta, no specific viral changes have been noted [14]. However, a recent small study [15] demonstrated that placentas positive for SARS-CoV-2 via RNA ISH demonstrated chronic histiocytic intervillositis, perivillous fibrin deposition, and trophoblastic necrosis. Interestingly, 5 of 8 (63%) of the infants in this study [15] tested negative for SARS-CoV-2, meaning that the placenta may be infected while the infant is not in a number of cases.

2. Materials and methods

2.1. Study design

This was a retrospective observational consecutive cohort study of mothers with a positive SARS-CoV-2 RT-PCR result during their current pregnancy who delivered at The University of North Carolina (UNC) Hospitals in Chapel Hill, NC, between December 1, 2019 and May 31, 2021, and whose placentas were evaluated by Anatomic Pathology. Their infants were included in the cohort. We excluded mothers and their infants who were not the product of a singleton birth and infants found to have documented genetic abnormalities. Data was obtained from the electronic medical record (EMR) in accordance with the University of North Carolina at Chapel Hill Internal Review Board-approved study parameters (IRB# 20-2944).

2.2. COVID-19 severity quantification

We utilized a simplification of the WHO ordinal scale [16] to categorize the maternal COVID-19 severity. The WHO ordinal scale classifies COVID-19 severity into 5 major categories: uninfected, ambulatory mild disease, hospitalized moderate disease, hospitalized severe disease, or dead with an associated numerical score from 0 to 10 depending on the severity of symptoms (0 for uninfected up to 10 for dead). In the WHO ordinal scale, ambulatory mild disease consists of asymptomatic patients with viral RNA detected, symptomatic and independent patients, or symptomatic patients who need home assistance. For simplification, we divided ambulatory mild disease into SARS-CoV-2-infected asymptomatic patients who were assigned a score of 1 and those who were symptomatic and were either independent or needed assistance at home as mild and assigned these patients a score of 2. Patients hospitalized for COVID-19 complications, who either did not need oxygen therapy or received oxygen by mask or nasal prongs at any point of their hospitalization were classified as moderate and assigned a score of 3. Patients that were hospitalized for COVID-19 complications and required oxygen via high flow or non-invasive ventilation, mechanical ventilation with or without vasodilators, dialysis, or required extracorporeal membrane oxygenation (ECMO) were categorized as severe and assigned a score of 4.

2.3. Newborn weight, length, and head circumference percentiles

Newborn weight, length, and head circumference percentiles were based on the WHO 0-2-year-old growth charts [17].

2.4. SARS-CoV-2 serologic testing

In a smaller non-consecutive study, from July 10, 2021 to August 31, 2021, we identified 10 mothers who had a positive SARS-CoV-2 RT-PCR during their current pregnancy, had not received a COVID-19 vaccination, delivered their infants at UNC Hospital main campus, and both the mother and their infant had enough blood remaining in the laboratory to perform SARS-CoV-2-specific antibody assays. SARS-CoV-2-specific IgG antibodies against the spike tetramer, S1, RBD, S2, and nucleocapsid proteins were detected from patient serum or plasma utilizing the Lab-Screen COVID plus multiplex bead assay (OneLambda) per manufacturer instructions on the LabScan3D platform (Luminex). These same samples, in addition to appropriate positive and negative controls, were tested for SARS-CoV-2 neutralizing antibodies using a surrogate neutralizing antibody assay (GeneScript) per the manufacturer's instructions.

2.5. Placenta processing and examination

The placentas were processed and evaluated using standard methods [18]. All noted gross and histologic findings were quantified from the final pathology reports in the EMR. The placentas were assigned to one (or more) of the five Amsterdam consensus group criteria or classified as normal/non-threshold if the placental findings did not meet the diagnostic criteria for one of these classifications [2].

2.6. Immunohistochemical and RNAScope analysis

Immunohistochemistry for SARS coronavirus nucleocapsid (Invitrogen PA1-41098) and RNA in situ hybridization for SARS-CoV-2 RNA (Advanced Cell Diagnostics, 848561) in selected placental tissues were performed as previously described including appropriate positive and negative controls [19,20].

2.7. Statistical analysis

All statistical analyses were performed using GraphPad Prism version 9.2.0 except for polynomial and linear trend analysis, which were performed using Microsoft Excel (Microsoft 365). Chi-square for linear trend, Fischer exact test, Mann-Whitney test, or one-way ANOVA with multiple comparisons between groups using Tukey's test were used as appropriate. Survival curve comparison was performed using the Gehan-Breslow-Wilcoxon test. P values < 0.050 were considered significant.

3. Results

3.1. Maternal characteristics

One hundred twenty-five mothers were identified who were infected with SARS-CoV-2 during their pregnancy. For simplicity in comparison, we excluded twin gestations (n = 4). Since we wanted to determine if SARS-CoV-2 had any effect on neonatal development and led to any congenital abnormalities, we excluded 6 mothers with infants that were found to have chromosomal or genetic abnormalities. Therefore, the final cohort included 115 mothers and their infants who met the inclusion criteria for our study (Fig. 1).

Clinical information that was collected and analyzed is listed in Table 1. The mean (median) maternal age was 29 (28) years with a range of 15–42 years old. 58% of the mothers were Hispanic, which is substantially increased compared to 9.6%, the estimated Hispanic statewide

Mothers Who Were SARS-CoV-2 Positive via PCR-Based Assay At Any Point of Their Pregnancy From December 1, 2019 to May 31, 2021
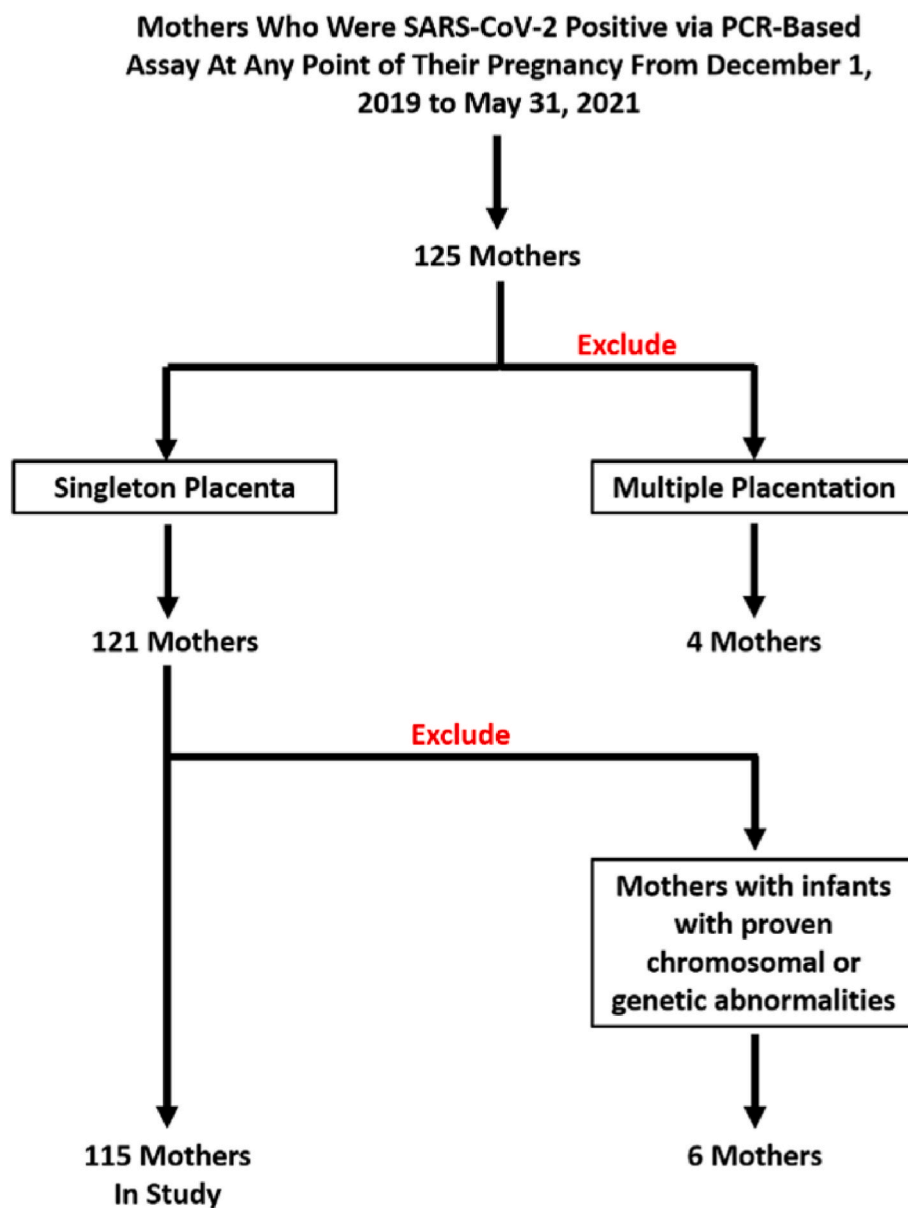

Fig. 1. Flow diagram of selection of study cohort.

population of North Carolina [21]. Nine mothers (7.83%) were infected with SARS-CoV-2 in their first trimester, 27 (23.5%) were infected in their second trimester, with the remaining 79 (68.7%) infected in their third trimester. The mean (median) of the first positive RT-PCR result was 30.5 (34.6) weeks gestation, and ~35% tested positive on admission. The average (median) gestational age at delivery was 38.3 (39 1/7) weeks with 69% vaginal deliveries and 31% via C-section. 85.2% of mothers were asymptomatic (n = 37) or had mild (n = 61) symptoms, 13.0% had moderate (n = 9) or severe (n = 6) COVID-19, and 1.74% (n = 2) did not have their symptoms reported.

3.2. Severity of maternal COVID-19 infection

Mothers with severe or moderate COVID-19 were statistically more likely to be Type 1 (p = 0.0055) or Type 2 (p = 0.0285) diabetics, deliver via C-section (p = 0.0006), have any delivery complication (p = 0.0291), preeclampsia (p = 0.0274), or premature infants (p = 0.0002) compared to mothers who were mildly ill (less likely) or asymptomatic (least likely). Three of the six severely ill mothers delivered at 26-32 weeks and one severely ill and two of the nine moderately ill mothers

delivered at 35-36 weeks due to respiratory failure. 8.70% of mothers required supplemental oxygen with respiratory failure attributed to COVID-19 in 7 mothers (6.09%). Mothers with severe COVID-19 delivered prematurely, at an average (median) gestational age of 33.6 (34 1/7) weeks compared to 38.5 (39 0/7) weeks with asymptomatic infection (p < 0.0001), 38.7 (39 2/7) weeks with mild COVID-19 (p < 0.0001), and 37.5 (37 2/7) weeks with moderate COVID-19 (p = 0.0157). Subgroup analysis demonstrated a statistically significant difference in delivery complications between mild versus moderate (p = 0.0300) and mild versus severe (p = 0.0253) maternal infections. As expected, mothers who were moderately (p < 0.0001) or severely (p < 0.0001) ill were statistically more likely to be treated with medications for COVID-19, including high-dose corticosteroids, than asymptomatic or mildly ill mothers.

3.3. Trimester of maternal COVID-19 infection

There was a statistically significant increase in the maternal requirement for supplemental oxygen (p = 0.0393), incidence of C-section (p = 0.0012), presence of clinically noted meconium (p =

Table 1

Mother, infant, and placenta characteristics of the entire cohort, with comparison of these characteristics between the severity of COVID-19, and the trimester of SARS-CoV-2 infection.

	Entire Cohort (n = 115)	Asymptomatic COVID-19 (n = 37)	Mild COVID-19 (n = 61)	Moderate COVID-19 (n = 9)	Severe COVID-19 (n = 6)	First Trimester COVID-19 (n = 9)	Second Trimester COVID-19 (n = 27)	Third Trimester COVID-19 (n = 79)
Maternal Demographics								
Average (median) maternal age	29 (28)	30 (30)	28 (27)	28 (26)	32 (31)	27 (27)	28 (28)	29 (28)
Race/Ethnicity								
Latina/Hispanic	58.3% [67]	62.2% [23]	59.0% [36]	44.4% [4]	50.0% [3]	88.9% [8]	55.6% [15]	55.7% [44]
African American/African	17.4% [20]	13.5% [5]	23.0% [14]	11.1% [1]	0% [0]	0% [0]	22.2% [6]	17.7% [14]
White, Non-Hispanic	17.4% [20]	18.9% [7]	13.1% [8]	22.2% [2]	33.3% [2]	11.1% [1]	18.5% [5]	17.7% [14]
East Asian	1.74% [2]	0% [0]	1.64% [1]	0% [0]	16.7% [1]	0% [0]	0% [0]	2.50% [2]
Mixed heritage	2.61% [3]	2.70% [1]	3.28% [2]	0% [0]	0% [0]	0% [0]	3.70% [1]	2.50% [2]
No race/ethnicity available	2.61% [3]	2.70% [1]	0% [0]	22.2% [2]	0% [0]	0% [0]	0% [0]	4.00% [3]
Obstetrical Data								
Average (median) gestational age at delivery	38.3 (39 1/7) weeks	38.5 (39 0/7) weeks[d,e]	38.7 (39 2/7) weeks[d,f]	37.5 (37 2/7) weeks[d,g]	33.6 (34 1/7) weeks[d,e,f,g]	37.5 (38 5/7) weeks	38.68 (39 3/7) weeks	38.23 (39 0/7) weeks
First Pregnancy	26.1% [30]	18.9% [7]	27.9% [17]	44.4% [4]	16.7% [1]	44.4% [4]	18.5% [5]	26.6% [21]
Type of Delivery								
Vaginal	68.7% [79]	75.7% [28][ee,ff]	73.8% [45][ee,gg]	44.4% [4][ee]	0% [0][ee,ff,gg]	100% [9][s,t]	85.2% [23][s,u]	59.5% [47][s,t,u]
Cesarean Section	31.3% [36]	24.3% [9][ee,ff]	26.2% [16][ee,gg]	55.6% [5][ee]	100% [6][ee,ff,gg]	0% [0][s,t]	14.8% [4][s,u]	40.5% [32][s,t,u]
Maternal COVID-19 Data								
Average (median) SARS-CoV-2 Ct Value	26.6 (25.7)	33.7 (38.3)[a,b,c]	20.2 (19.5)[a,b]	21.6 (21.4)[a]	18.2 (17.2)[a,c]	21.0 (22.2)[q]	20.8 (20.7)[q]	28.7 (30.6)[q]
Average (median) gestational age at first SARS-CoV-2-positive test	30.5 (34 4/7) weeks	35.9 (38 6/7) weeks	26.76 (30 2/7) weeks	32.9 (34 4/7) weeks	31.97 (31.93) weeks	7.63 (8 6/7) weeks	20.39 (20 4/7) weeks	36.63 (37 4/7) weeks
Infection in first trimester	7.83% [9]	2.70% [1]	13.1% [8]	0% [0]	0% [0]	–	–	–
Infection in second trimester	23.5% [27]	8.11% [3]	36.1% [22]	11.1% [1]	0% [0]	–	–	–
Infection in third trimester	68.7% [79]	89.2% [33]	50.8% [31]	88.9% [8]	100% [6]	–	–	–
Infection ≤34 0/7 weeks of third trimester	16.5% [19]	5.41% [2]	14.8% [9]	11.1% [1]	83.3% [5]	–	–	–
Infection ≥34 1/7 weeks of third trimester	52.2% [60]	83.8% [31]	36.1% [22]	33.3% [3]	16.7% [1]	–	–	–
Medications	9.56% [11]	0% [0][tt,uu,vv]	0% [0][tt,ww,xx]	66.7% [6][tt,uu,ww]	83.3% [5][tt,vv,xx]	0% [0][yy]	0% [0][yy]	13.9% [11][yy]
Corticosteroids	6.09% [7]	–	–	44.4% [4]	50.0% [3]	–	–	8.86% [7]
Remdesivir	4.35% [5]	–	–	55.5% [5]	83.3% [5]	–	–	6.33% [5]
Bamlanivimab	1.74% [2]	–	–	11.1% [1]	16.7% [1]	–	–	2.53% [2]
Enoxaparin	1.74% [2]	–	–	11.1% [1]	16.7% [1]	–	–	2.53% [2]
COVID-19 Convalescent Plasma	0.87% [1]	–	–	–	16.7% [1]	–	–	1.27% [1]
Required treatment with oxygen	8.70% [10]	0% [0]	0% [0]	44.4% [4]	100% [6]	0% [0][r]	0% [0][r]	2.7% [10][r]
Pregnancy-related medical comorbidities								
Average (median) maternal BMI	30.30 (28.57)	29.83 (27.96)	30.59 (29.67)	33.04 (33.00)	28.97 (29.00)	28.73 (26.96)	31.79 (31.00)	29.93 (28.25)
Hypertension	22.6% [26]	16.2% [6]	26.2% [16]	33.3% [3]	16.7% [1]	11.1% [1]	22.2% [6]	24.1% [19]
Chronic	8.70% [10]	8.11% [3]	8.20% [5]	11.1% [1]	16.7% [1]	11.1% [1]	11.1% [3]	7.59% [6]
Gestational	13.9% [16]	8.11% [3]	18.0% [11]	22.2% [2]	0% [0]	0% [0]	11.1% [3]	16.5% [13]
Diabetes mellitus	14.8% [17]	13.5% [5]	11.5% [7]	33.3% [3]	33.3% [2]	11.1% [1]	7.41% [2]	17.7% [14]
Gestational	9.57% [11]	10.8% [4]	9.84% [6]	11.1% [1]	0% [0]	11.1% [1]	3.70% [1]	11.4% [9]
T1DM	0.87% [1]	0% [0][qq]	0% [0][qq]	0% [0][qq]	16.7% [1][qq]	0% [0]	0% [0]	1.27% [1]
T2DM	4.35% [5]	2.70% [1][rr]	1.64% [1][rr,ss]	22.2% [2][rr,ss]	16.7% [1][rr]	0% [0]	3.70% [1]	5.06% [4]
Current smoker during pregnancy	6.96% [8]	8.11% [3]	6.56% [4]	11.1% [1]	0% [0]	0% [0]	3.70% [1]	8.86% [7]
Birth Complications								
Any birth complication reported by clinical team	55.7% [64]	54.1% [20][hh]	47.5% [29][hh,ii,jj]	88.9% [8][hh,ii]	100% [6][hh,jj]	44.4% [4]	44.4% [12]	60.8% [48]

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Table 1 (*continued*)

	Entire Cohort (n = 115)	Asymptomatic COVID-19 (n = 37)	Mild COVID-19 (n = 61)	Moderate COVID-19 (n = 9)	Severe COVID-19 (n = 6)	First Trimester COVID-19 (n = 9)	Second Trimester COVID-19 (n = 27)	Third Trimester COVID-19 (n = 79)
Meconium	13.9% [16]	24.3% [9][kk]	9.84% [6][kk]	11.1% [1][kk]	0% [0][kk]	0% [0][v]	3.70% [1][v]	17.7% [14][v]
Chorioamnionitis	6.09% [7]	5.41% [2]	6.56% [4]	11.1% [1]	0% [0]	0% [0]	7.41% [2]	6.33% [5]
pPROM/PROM	2.61% [3]	5.41% [2]	1.64% [1]	0% [0]	0% [0]	0% [0]	0% [0]	3.80% [3]
Intrauterine growth restriction	3.48% [4]	8.11% [3]	1.64% [1]	0% [0]	0% [0]	0% [0]	0% [0]	5.06% [4]
Nuchal chord	11.3% [13]	16.2% [6]	11.5% [7]	0% [0]	0% [0]	0% [0]	11.1% [3]	12.7% [10]
Hemorrhage	12.2% [14]	13.5% [5]	11.5% [7]	11.1% [1]	16.7% [1]	11.1% [1]	14.8% [4]	11.4% [9]
Fetal intolerance	12.2% [14]	13.5% [5]	9.84% [6]	22.2% [2]	16.7% [1]	0% [0][w]	3.70% [1][w]	16.5% [13][w]
Failure to progress	7.83% [9]	5.41% [2]	9.84% [6]	11.1% [1]	0% [0]	0% [0]	7.40% [2]	8.86% [7]
Preeclampsia/HELLP syndrome	13.0% [15]	5.41% [2][ll, mm]	11.5% [7][ll, nn]	44.4% [4][ll, mm, nn]	16.7% [1][ll]	11.1% [1]	0% [0]	11.4% [9]
Elevated BP	0.87% [1]	2.70% [1]	0% [0]	0% [0]	0% [0]	0% [0]	0% [0]	1.27% [1]
Fever	2.61% [3]	0% [0]	3.28% [2]	11.1% [1]	0% [0]	0% [0]	0% [0]	3.80% [3]
Maternal respiratory failure	6.09% [7]	0% [0]	0% [0]	11.1% [1]	100% [6]	0% [0]	0% [0]	8.86% [7]
Medical maternal complications	2.61% [3]	0% [0][oo]	1.64% [1][pp]	22.2% [2][oo, pp]	0% [0]	11.1% [1]	0% [0]	2.53% [2]
Severe intrahepatic cholestasis of pregnancy	0.87% [1]	–	1.64% [1]	–	–	11.1% [1]	–	–
DKA/starvation ketosis in the setting of T2DM and symptomatic COVID-19	0.87% [1]	–	–	11.1% [1]	–	–	–	1.27% [1]
Cholestasis of pregnancy	0.87% [1]	–	–	11.1% [1]	–	–	–	1.27% [1]
GBS positive	6.96% [8]	2.70% [1]	6.56% [4]	11.1% [1]	16.7% [1]	33.3% [3][x,y,z]	3.71% [1][x,y]	5.06% [4][x,z]
Preterm labor	0.87% [1]	0% [0]	1.64% [1]	0% [0]	0% [0]	11.1% [1][aa]	0% [0][aa]	0% [0][aa]
Abruptio placenta	0.87% [1]	0% [0]	1.64% [1]	0% [0]	0% [0]	11.1% [1][bb]	0% [0][bb]	0% [0][bb]
Retained placenta	0.87% [1]	0% [0]	1.64% [1]	0% [0]	0% [0]	0% [0]	3.71% [1]	0% [0]
Oligohydramnios	0.87% [1]	2.70% [1]	0% [0]	0% [0]	0% [0]	0% [0]	0% [0]	1.27% [1]
Shoulder dystocia	0.87% [1]	0% [0]	1.64% [1]	0% [0]	0% [0]	0% [0]	3.71% [1]	0% [0]
Newborn Data								
Newborn COVID-19 Data								
Newborns tested for SARS-CoV-2	59.1% [68]	89.2% [33]	39.3% [24]	66.7% [6]	83.3% [5]	0% [0][cc]	22.2% [6][cc, dd]	78.5% [62][cc, dd]
Positive	1.47% [1]	3.03% [1]	0% [0]	0% [0]	0% [0]	–	0% [0]	1.61% [1]
Negative	98.5% [67]	97.0% [32]	100% [24]	100% [6]	100% [5]	–	100% [6]	98.4% [61]
Newborn Characteristics								
Average (median) newborn weight	3142 (3235) g	3099 (3250) g	3210 (3245) g	3380 (3135) g	2394 (2590) g	3050 (3240) g	3383 (3325) g	3140 (3175) g
Average (median) newborn length	49.1 (50.8) cm	48.7 (49.5) g	49.6 (50.8) cm	51.0 (51.8) cm	45.0 (45.1) cm	49.8 (50.8) cm	50.2 (50.8) cm	48.7 (50.0) cm
Average (median) newborn head circumference	34.0 (34.3) cm	33.5 (34.3) g	34.5 (34.3) cm	34.9 (34.9) cm	30.9 (32.0) cm	33.4 (34.3) cm	34.2 (34.5) cm	34.0 (34.3) cm
Average (median) APGAR scores								
1 min	7.5 (8.0)	7.5 (8.0)	7.9 (8.0)	5.9 (6.5)	6.3 (7.0)	8.0 (8.0)	7.7 (8.0)	7.4 (8.0)
5 min	8.6 (9.0)	8.8 (9.0)	8.7 (9.0)	8.0 (8.5)	8.0 (9.0)	8.8 (9.0)	8.7 (9.0)	8.6 (9.0)
NICU/PICU admission	20.0% [23]	18.9% [7][h,i,j]	9.84% [6][h,k,l]	55.6% [5][h,i,k]	83.3% [5][h, j,l]	11.1% [1]	7.41% [2]	25.3% [20]
Newborn Complications								
Premature (<37 weeks)	12.2% [14]	10.8% [4][m,n]	4.92% [3][m,o]	22.2% [2][m,p]	83.5% [5][m,n,o,p]	11.1% [1]	7.41% [2]	13.9% [11]
Large for gestational age	6.96% [8]	5.41% [2]	6.56% [4]	22.2% [2]	0% [0]	0% [0]	7.41 [2]	7.59% [6]
Small for gestational age	3.48% [4]	8.11% [3]	1.64% [1]	0% [0]	0% [0]	0% [0]	0% [0]	5.06% [4]
Newborn Physical Abnormalities								
Newborns with any physical abnormality	13.0% [15]	13.5% [5]	11.5% [7]	22.2% [2]	16.7% [1]	0% [0]	18.5% [5]	12.7% [10]
Sacral dimple	2.61% [3]	2.70% [1]	1.64% [1]	0% [0]	16.7% [1]	–	3.70% [1]	2.53% [2]
Cardiovascular abnormality	1.74% [2]	–	3.28% [2]	–	–	–	3.70% [1]	1.27% [1]
Spina bifida occulta with sacral hair tuft	0.87% [1]	–	1.64% [1]	–	–	–	3.70% [1]	–
Bilateral hydroceles	0.87% [1]	–	1.64% [1]	–	–	–	3.70% [1]	–
Transverse palmar crease and widened toe gap	0.87% [1]	–	1.64% [1]	–	–	–	3.70% [1]	–
Congenital phalangeal synostosis bilateral 4th and 5th PIP joints of the hand	0.87% [1]	–	–	11.1% [1]	–	–	–	1.61% [1]
Mild micrognathia	0.87% [1]	–	–	11.1% [1]	–	–	–	1.61% [1]
Right ear tag, caput molding, and diastasis recti	0.87% [1]	–	1.64% [1]	–	–	–	–	1.61% [1]
Mild deviation of the penile raphe	0.87% [1]	–	1.64% [1]	–	–	–	3.70% [1]	–

Table 1 (*continued*)

	Entire Cohort (n = 115)	Asymptomatic COVID-19 (n = 37)	Mild COVID-19 (n = 61)	Moderate COVID-19 (n = 9)	Severe COVID-19 (n = 6)	First Trimester COVID-19 (n = 9)	Second Trimester COVID-19 (n = 27)	Third Trimester COVID-19 (n = 79)
Low set ears and prominent epicanthal fold concerning for Trisomy 21 (with normal karyotype)	0.87% [1]	2.70% [1]	–	–	–	–	–	1.61% [1]
Scaphocephalic head	0.87% [1]	2.70% [1]	–	–	–	–	–	1.61% [1]
Newborn Screening Tests								
Failed first hearing screen	4.35% [5]	5.41% [2]	3.28% [2]	11.1% [1]	0% [0]	0% [0]	3.70% [1]	5.1% [4]
Failed follow-up hearing testing	1.74% [2]	0% [0]	1.64% [1]	11.1% [1]	–	–	3.70% [1]	1.27% [1]
Passed congenital heart screen	100% [115]	100% [37]	100% [61]	100% [9]	100% [6]	100% [9]	100% [27]	100% [79]
Placenta Characteristics								
Amsterdam Criteria Classification								
Maternal vascular malperfusion	22.6% [26]	27.0% [10]	18.0% [11]	22.2% [2]	33.3% [2]	0% [0]	22.2% [6]	25.3% [20]
Fetal vascular malperfusion	0.87% [1]	0% [0]	1.64% [1]	0% [0]	0% [0]	0% [0]	0% [0]	1.27% [1]
Delayed villous maturation	0.87% [1]	2.70% [1]	0% [0]	0% [0]	0% [0]	0% [0]	0% [0]	1.27% [1]
Ascending intrauterine infection	24.3% [28]	13.5% [5]	31.1% [19]	33.3% [3]	0% [0]	44.4% [4]	14.8% [4]	25.3% [20]
Villitis of unknown etiology	3.48% [4]	2.70% [1]	3.28% [2]	11.1% [1]	0% [0]	0% [0]	0% [0]	5.06% [4]
Normal or non-threshold findings	54.8% [63]	56.8% [21]	54.1% [33]	44.4% [4]	66.7% [4]	55.6% [5]	70.4% [19]	49.4% [39]
Average (median) placenta weight	459 (450) g	445 (440) g	467 (450) g	502 (495) g	389 (373) g	453 (425) g	458 (450) g	460 (450) g
Average (median) fetal weight to placenta weight ratio	7.00 (6.98)	7.13 (6.86)	7.01 (7.12)	6.93 (7.04)	6.39 (6.41)	6.77 (6.71)	7.30 (7.26)	6.92 (6.89)

[a] $p < 0.0001$, [b] $p < 0.0001$, [c] $p = 0.0339$, [d] $p < 0.0001$, [e] $p < 0.0001$, [f] $p < 0.0001$, [g] $p = 0.0157$, [h] $p = 0.0007$, [i] $p = 0.0388$, [j] $p = 0.0042$, [k] $p = 0.0035$, [l] $p = 0.0003$, [m] $p = 0.0002$, [n] $p = 0.0007$, [o] $p < 0.0001$, [p] $p = 0.0406$, [q] $p = 0.0341$, [r] $p = 0.0393$, [s] $p = 0.0012$, [t] $p = 0.0234$, [u] $p = 0.0182$, [v] $p = 0.024$, [w] $p = 0.0421$, [x] $p = 0.0240$, [y] $p = 0.0406$, [z] $p = 0.0215$, [aa] $p = 0.0101$, [bb] $p = 0.0101$, [cc] $p < 0.0001$, [dd] $p < 0.0001$, [ee] $p = 0.0006$, [ff] $p = 0.008$, [gg] $p = 0.0007$, [hh] $p = 0.0291$, [ii] $p = 0.0300$, [jj] $p = 0.0253$, [kk] $p = 0.0457$, [ll] $p = 0.0274$, [mm] $p = 0.0095$, [nn] $p = 0.0289$, [oo] $p = 0.0304$, [pp] $p = 0.0417$, [qq] $p = 0.0055$, [rr] $p = 0.0285$, [ss] $p = 0.0417$, [tt] $p < 0.0001$, [uu] $p < 0.0001$, [vv] $p < 0.0001$, [ww] $p < 0.0001$, [xx] $p < 0.0001$, and [yy] $p = 0.0298$.

0.0241), and fetal intolerance (p = 0.042) when progressing from maternal SARS-CoV-2 infection in the first trimester (least risk) to the third trimester (most risk). There was a statistically significant increased odds ratio (OR 3.91, 1.31–11.2, 95% CI) of C-section with a third trimester maternal SARS-CoV-2 infection versus a first trimester (p = 0.0234) or a second trimester (p = 0.0182) infection. Conversely, there was a statistically significant increase in group B *Streptococcus* (GBS) infection (p = 0.0240), preterm labor (p = 0.0101), and *abruptio placentae* (p = 0.0101) from first trimester (most risk) to third trimester (least risk) maternal infection.

3.4. Infant characteristics

In the cohort, there were more male infants than female infants (Fig. 2A). Infant birthweights (Fig. 2B), lengths (Fig. 2C), and head circumferences (Fig. 2D) were all within normal parameters for gestational age. On subgroup analysis, there was a statistically significant decrease in the mean infant weights with severe versus mild (p = 0.0246) or moderate (p = 0.0286) maternal COVID-19. There was a statistically significant decrease in the mean infant length (p = 0.0372) with moderate or severe maternal infections. Similarly, there was a statistically significant decrease in infant head circumferences with severe maternal infections versus mild infections (p = 0.0428). Overall, these measurements correlate with the increased premature births in moderately or severely symptomatic mothers.

Twenty-three (20.0%) of the infants were admitted to the neonatal intensive care unit (NICU) after birth. A severe maternal infection was associated with an increased incidence of admission to the NICU (p = 0.0007) and premature birth (p = 0.0002). Subgroup analysis showed significantly increased admission to the NICU after moderate (p = 0.0388) or severe (p = 0.0042) versus asymptomatic maternal infections. Similarly, mild versus moderate (p = 0.0035) and mild versus severe (p = 0.0003) maternal COVID-19 were associated with an increased risk of NICU admission. Also, asymptomatic versus severe (p

= 0.0007) and mild versus severe (p < 0.0001) maternal COVID-19 were associated with a statistically increased risk of premature birth. Despite prematurity and/or NICU admissions, all infants were alive at the completion of the study on May 31, 2021 and there were no incidents of fetal demise.

Fifteen (13.0%) infants had a reported physical abnormality (Table 1). The most common was a deep sacral dimple in three infants (2.61%). However, there were no major or recurring physical abnormalities seen in any of the infants, including those born after a first or second trimester maternal SARS-CoV-2 infection. Five (4.3%) infants failed their newborn hearing screen. Of these 5 infants, 3 passed on follow up hearing tests, and 2 infants failed again (1.7%). There was insufficient follow up in these two infants as to the cause of hearing loss; however, there was no indication that hearing loss was secondary to maternal SARS-CoV-2 infection. All infants were screened for congenital heart disease via pulse oximetry and 100% passed, with 2 infants later found to have completely dissimilar cardiovascular abnormalities (one with a family history of congenital heart disease in the father).

3.5. Maternal transmission

Sixty-eight infants (59.1%) were tested for SARS-CoV-2 RNA at 24 h of life and 40 of the infants (34.8%) received a second test at 48 h of life. Of these 68 infants, only one infant (1.47%) was found to be positive for SARS-CoV-2 by RT-PCR at 24 h. If we assume that the untested infants were also negative, the overall incidence of SARS-CoV-2 infection was 0.870% in our cohort. Many of the infants stayed in the same rooms with their mothers and nearly all the infants were breastfed. There was no evidence of placental SARS-CoV-2 infection in the infant that tested positive, meaning that transmission from the mother to infant was likely not via the placenta (Fig. 3). Based on these findings, we hypothesized that the lack of SARS-CoV-2 infection in the infants was due to vertical transfer of SARS-CoV-2-specfic IgG antibodies.

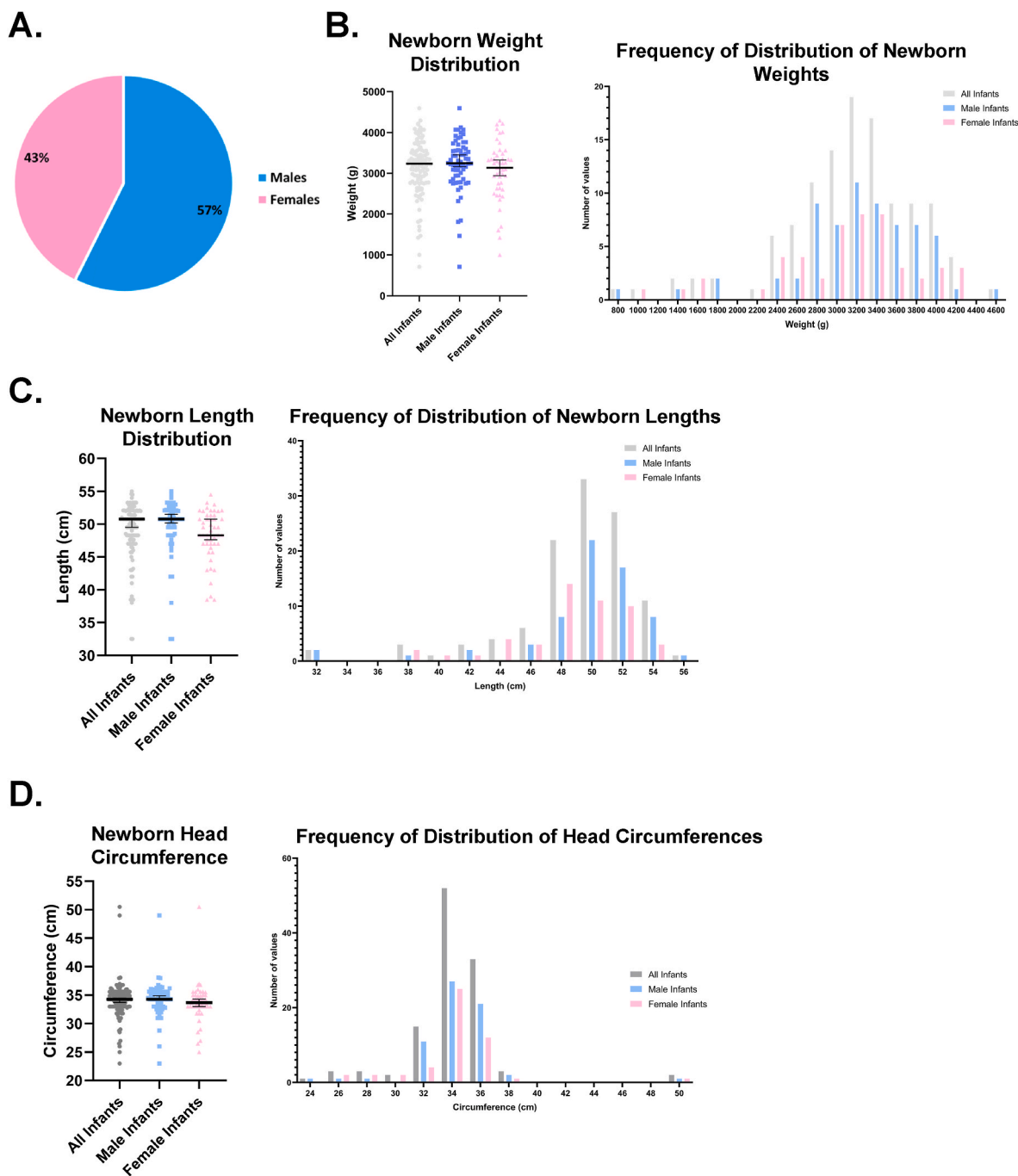To test this hypothesis, we performed a smaller non-consecutive

Fig. 2. Sex, weight, length, and head circumference distribution of infants within the study cohort.

study, from July 10, 2021 to August 31, 2021, where we identified 10 mothers who had a positive SARS-CoV-2 RT-PCR during their current pregnancy, had not received a COVID-19 vaccination, delivered their infants at UNC Hospitals main campus, and both the mother and their infant had enough blood remaining in the laboratory to perform SARS-CoV-2-specific antibody assays. Nine of 10 mothers (90%) had SARS-CoV-2-specific IgG antibodies and these antibodies were detected in the blood of their infants. One mother (10%) did not develop a significant antibody response to her SARS-CoV-2 infection. The mother was a type II diabetic who was hospitalized over a month for a foot ulcer and septicemia. She contracted SARS-CoV-2 in the hospital several days before delivery. Her infant tested positive for SARS-CoV-2 by RT-PCR and did not have any detectable SARS-CoV-2 IgG antibodies. Similar confirmatory results were found with a surrogate assay for SARS-CoV-2

neutralizing antibodies (Fig. 4).

3.6. Placental pathology

54.8% of the placentas had normal findings or did not meet the diagnostic criteria for any of the five placental patterns of injury as described in the Amsterdam consensus statement (Fig. 5A) [2]. The remaining placentas demonstrated maternal vascular malperfusion (MVM, 22.6%), ascending intrauterine infection (AIUI, 24.3%), villitis of unknown etiology (3.48%), fetal vascular malperfusion (FVM, 0.870%), and/or delayed villous maturation (0.870%). There was no statistically significant difference in the incidence of any of the Amsterdam patterns of placental injury among the severity groups (asymptomatic to severe) or the trimester of maternal infection.
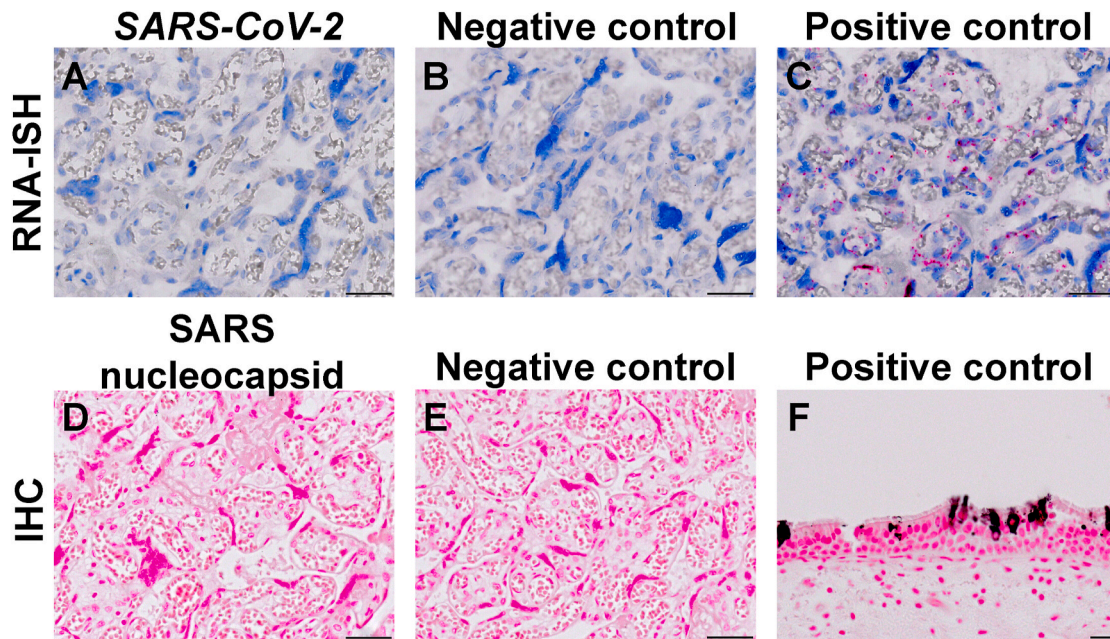
Fig. 3. RNAScope for SARS-CoV-2 and immunohistochemistry for SARS-CoV-2 spike protein demonstrate the absence of placental infection with SARS-CoV-2 in the single SARS-CoV-2 positive infant in our cohort. (A–C) RNA-ISH of placenta for *SARS-CoV*-2 RNA (A) and negative (bacterial *DapB*) (B) and positive (*UBC*) (C) controls. (D–E) IHC of placenta for SARS nucleocapsid (D) and isotype control (E). (F) IHC of COVID-19 trachea as a positive control. Bars: (A–E): 50 μm. (F) 20 μm.
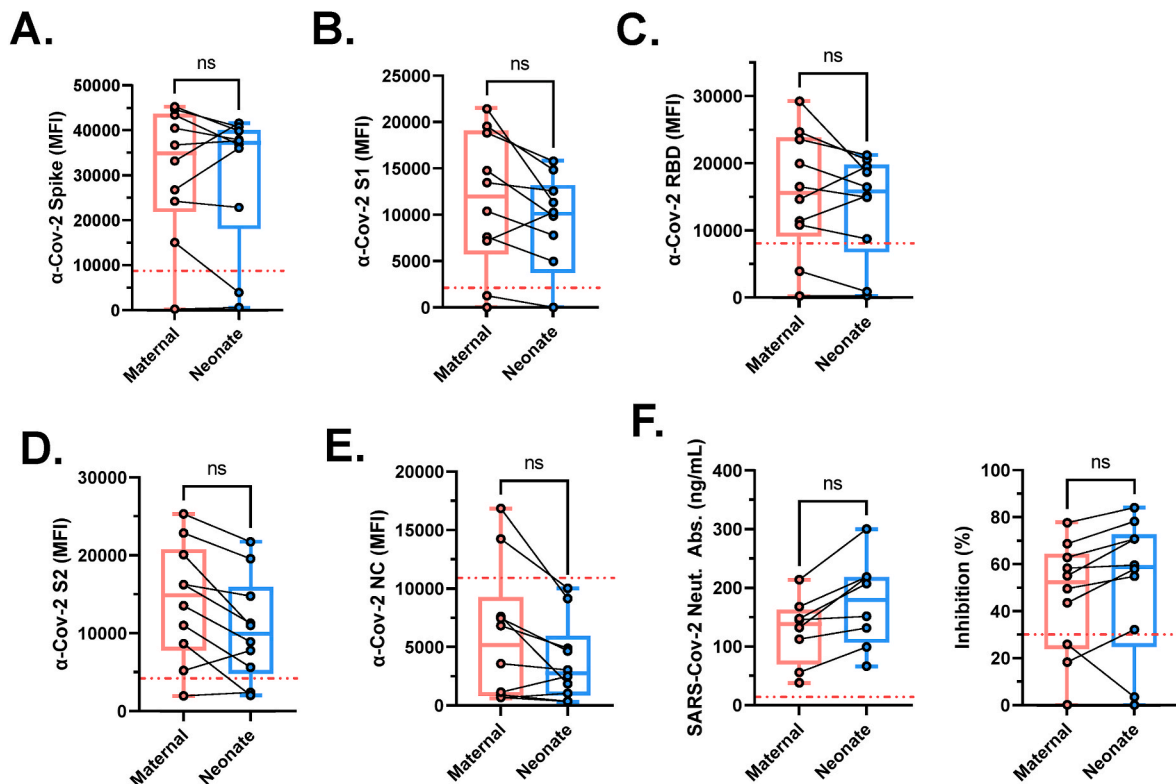

Fig. 4. SARS-CoV-2-specific IgG antibodies in mothers and their infants. Box and whisker plots demonstrating **A:** SARS-CoV-2 spike IgG antibody, **B:** SARS-CoV-2 spike glycoprotein S1 subunit IgG antibody, **C:** SARS-CoV-2 receptor-binding domain IgG antibody, **D:** SARS-CoV-2 spike glycoprotein 2 subunit IgG antibody, and **E:** SARS-CoV-2 Nucleocapsid IgG antibody. **F:** SARS-CoV-2-surrogate neutralizing IgG antibody concentration and percent inhibition. The dashed red line is the threshold for a positive test with each respective antibody assay. ns = not significant. n = 10.

We performed a subgroup analysis of the placentas with MVM by eliminating cofounding causes, including preterm delivery (<37 0/7-weeks), maternal diabetes mellitus, hypertension, and/or pre-eclampsia. The incidence of MVM decreased from 22.6% (n = 26) to 13.6% (n = 9), which was not statistically significant (p = 0.174). We also performed an analogous subgroup analysis of BMI (p = 0.563),
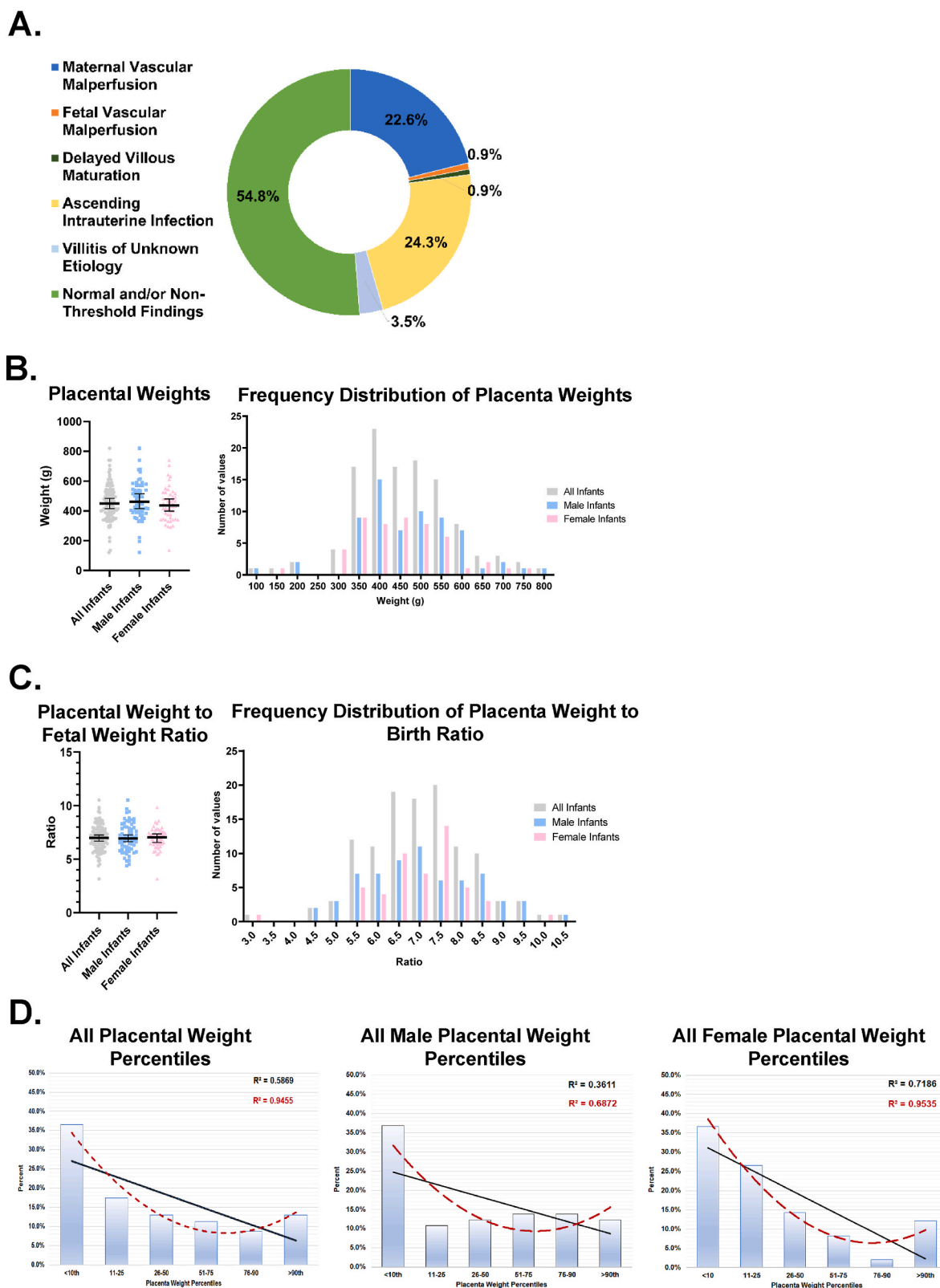
Fig. 5. Amsterdam criteria, placental weight, placental weight to birth weight ratio, and placental weight percentiles of placentas within the study cohort. A: Amsterdam criteria classification of the evaluated placentas. Maternal vascular malperfusion: n = 26, 22.6%; Fetal vascular malperfusion: n = 1, 0.870%; Delayed villous maturation: n = 1, 0.870%; Ascending intrauterine infection: n = 28, 24.3%; Villitis of unknown etiology: n = 4, 3.48%; Normal and/or non-threshold findings: n = 63, 54.8%. **B–C:** Scatter dot plot and bar graph of individual distribution of placental weights **(B)** and placental weight to birth weight ratio **(C)**. Solid line of scatter dot plot represents median with error bars representing 95% confidence intervals. **D:** Placental weight percentiles [36] for all placentas of male and female infants. Solid black line represents the linear trend line. Dashed red represents the 2nd order polynomial trend line.

maternal age (p = 0.769), vaginal birth versus C-section (p = 0.171), and supplemental oxygen requirement (p = 0.382), all of which were not statistically significant. Overall, none of the above clinical associations with MVM explained the increase in MVM in the cohort groups, suggesting that SARS-CoV-2 may be a contributing factor.

There was not a statistically significant difference (p = 0.454) between the male and female placental weights (Fig. 5B). The mean (median) placenta weight to birth weight ratio was 7.00 (6.98), 7.03 (6.96) for males, and 6.96 (7.04) for females, which was not statistically different (p = 0.948) (Fig. 5C). Interestingly, the placental weights were skewed toward lower percentiles, with 36.5% (36.9% of the males and 36.7% of the females) being less than the 10th percentile (Fig. 5D), but this was not directly correlated with COVID-19 severity or trimester of the SARS-Co2 infection.

4. Discussion

To date, many of the studies on SARS-CoV-2 and its effect on the mother, infant, and placenta [8,22] have been in late third trimester infections. Due to the length of time for our study, we were able to include a significant proportion of mothers who contracted SARS-CoV-2 in their first or second trimester. Moreover, we were able to follow these infants, in some cases over a year, for congenital birth defects and/or hearing impairment. In our cohort, there was no evidence of infant hearing loss or cardiovascular abnormalities conclusively linked to maternal SARS-CoV-2 infection. There was only one incident of SARS-CoV-2 transmission between a mother and infant (0.870%), for which we found no evidence of placental transmission. There was no fetal demise, and all the infants and mothers were alive at the end of the study. No significant physical or growth abnormalities were identified in any of the infants, including those from first or second trimester maternal COVID-19 infections, which is in keeping with a recent imaging study [23] that found no fetal growth restriction in SARS-CoV-2 infection during pregnancy. Importantly, we found mothers with moderate or severe maternal infections (13%) were statistically more likely to have preexisting Type 1 or Type 2 diabetes mellitus, and to have multiple and/or more severe maternal complications including pre-eclampsia, C-section and preterm delivery. Twenty percent (n = 23) of the infants in our study were admitted to the NICU, with most of these delivered by mothers with moderate or severe COVID-19. Finally, we found differences in pregnancy complications among the trimesters of infection. Group B *Streptococcus* (GBS) infection, preterm labor, and *abruptio placentae* were significantly higher in the first trimester infections. Conversely, the incidence of C-section, presence of clinically noted meconium, and fetal intolerance were significantly higher in the third trimester infections.

In our study, the incidence of maternal transmission was very low (0.870%) and was not via the placenta. After finding that only one infant in our original cohort became infected with SARS-CoV-2, we looked at a separate small cohort of mothers (n = 10) and their infants (n = 10) to determine if mothers infected with SARS-CoV-2 developed SARS-CoV-2-specific IgG antibodies and if these IgG antibodies were transferred to their newborns. We found that nearly all mothers (n = 9; 90%), except for one (n = 1; 10%), developed SARS-CoV-2-specifc IgG antibodies and transferred all or most of these antibodies to their infants. The mother who did not generate or transfer maternal antibodies may have had sepsis-induced immunosuppression from a very recent bacterial infection. The transmission of maternal antibodies likely accounts for the low incidence of SARS-CoV-2 infection in infants during pregnancy and the puerperium. This finding is in keeping with several recent studies [24–26] that demonstrated the maternal generation and vertical transmission of SARS-CoV-2-specific IgG antibodies. Our study adds to these studies by demonstrating that at least one of six SARS-CoV-2-specifc IgG antibodies we tested were transferred and retained by the infant and that this was irrespective of the trimester of the maternal SARS-CoV-2 infection.

A strength of our study was its relatively large size and its duration, which allowed us to identify first and second trimester maternal SARS-CoV-2 infections and to follow maternal and infant outcomes. Another potential strength of our study was that most of the mothers (~90%) in our cohort did not receive specific therapy for SARS-CoV-2, and none of the mothers were vaccinated, allowing our study to characterize the natural course of maternal SARS-CoV-2 infection on the placenta, mother, and infant. However, a limitation of the study was the lack of truly normal control placentas. The placentas received for pathologic examination at UNC are ordered by clinicians for known or suspected maternal, placental, or fetal conditions and are not sent from uncomplicated deliveries. Our study also lacked blinding of the pathologist to the maternal diagnosis of SARS-CoV-2 infection, which may present a risk for bias. However, since our results are similar to previously published studies [8,18,27], it is likely that this potential bias did not significantly affect the placental examination. Finally, another limitation of our study is that only 6 patients with severe COVID-19 were identified, meaning that the conclusions from these patients lacks significant statistical power. However, most studies to date have shown that severe symptomology is relatively uncommon [9,18]. Thus, a multiple institutional study will likely be required for severely ill COVID-19 mothers, their infants, and placentas to have significant statistical power.

Recently, it has been shown [28–32] that maternal transmission of SARS-CoV-2 during pregnancy is a rare event, which was confirmed in our current study. A recent review [33] found that breastfeeding, mother-to-infant contact, and the method of delivery did not increase the risk for neonatal SARS-CoV-2 infection. A possible reason for this protection is vertical transfer of SARS-CoV-2-specfic antibodies from the mother to the infant. In support of this hypothesis, several recent studies [24–26] have demonstrated the vertical transfer of SARS-CoV-2-specific IgG antibodies. Interestingly, these studies report an efficiency of transfer of these antibodies as low as 25% [26] and as high as 87% [24]. However, these studies characterized the vertical transfer of antibodies against a single SARS-CoV-2 epitope. Our study was able to simultaneously characterize the transfer of six different SARS-CoV-2-specific IgG antibodies with disparate epitopes in mother/infant dyads. While we found that not all these antibodies were successfully transferred from the mother to the infant, when looking at the transfer at least one antibody, the overall efficiency of transfer was 100%. This difference in placental antibody transfer efficiency of six different SARS-CoV-2-specific IgG antibodies may explain the reduced transplacental antibody transfer reported in previous studies [25,26] and the neonate protection despite the inefficient transfer of some antibodies. In support of this hypothesis, in the one mother in our antibody transmission study who did not develop any antibody response to SARS-CoV-2, her infant became infected with SARS-CoV-2. This suggests that the complete absence of maternal generation of SARS-CoV-2-specific IgG antibodies increases the risk of infection of the infant by the mother. Therefore, testing mothers and/or infants for these SARS-CoV-2 IgG antibodies may be warranted and considered as a part of the discussion with a mother deciding whether to room in with her infant while infected with SARS-CoV-2. Nevertheless, additional studies on the transfer and duration of the protective function of these SARS-CoV-2-specific antibodies are needed.

Consistent with previous reports [8,14,18], over half of the placentas in our study had no significant gross or microscopic abnormalities that met diagnostic criteria for a pattern of placental injury as defined by the Amsterdam consensus statement [2]. A minority of the placentas demonstrated MVM (22.6%) and/or AIUI (24.3%), and these placental findings were typically described as mild. Notably, the average placental weight to newborn birth weight ratio was in the normal range, indicating most placentas did not have significant functional abnormalities. This finding was consistent with most of the infants being of normal weight and size with no definitive abnormalities at birth. However, this study was conducted before the emergence of the Delta and Omicron

COVID-19 variants in North Carolina. A recent report [34] suggests that the Delta variant may be associated with more adverse fetal outcomes and more specific placental pathology. Therefore, future studies will be needed to determine if there are differences in placental findings and maternal and fetal outcomes among the SARS-CoV-2 variants.

There has been some speculation that maternal hypoxia associated with maternal COVID-19 infection may lead to maternal vascular malperfusion (MVM) [8]. While we found that a subset of placentas demonstrated MVM with weights below the 10th percentile, this was not linked to the severity of the maternal COVID-19 infection nor to known clinical associations with MVM such as hypertension, preeclampsia, and maternal diabetes. Therefore, future studies will be needed to determine if there are specific circumstances (e.g., underlying genetic factors, specific immune responses, particular SARS-CoV-2 variants, etc.), along with the timing of the maternal SARS-CoV-2 infection that correlate with the development of MVM and significantly decreased placental weight.

Fortunately, most mothers with SARS-CoV-2 infections acquired during pregnancy delivered at term and had uncomplicated vaginal deliveries. 31% of the deliveries in our cohort were by C-section, which is the national average for cesarean delivery in the United States [35]. However, moderate or severe COVID-19 maternal infection had an incidence of C-section of 55.6% (n = 5) and 100% (n = 6), respectively. In addition, pre-existing maternal diabetes mellitus was found to be a significant risk factor for developing moderate or severe maternal COVID-19 in our cohort, indicating currently pregnant women or women planning to become pregnant with diabetes mellitus should be encouraged to be vaccinated in order to prevent moderate to severe COVID-19 and its associated complications of delivery.

Disclosure of interests

The authors declare no conflicts of interest.

Contribution to authorship

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