UNIVERSIDADE DE BRASÍLIA

HOSPITAL UNIVERSITÁRIO DE BRASÍLIA

HUB UNB EBSERH

PROGRAMA DE RESIDÊNCIA MÉDICA EM PATOLOGIA

DÉBORA LUIZA ALBANO FULGENCIO

STUDY BETWEEN THE EFFECTS OF SARS-COV-2 INFECTION AND PLACENTAL MORPHOLOGY FEATURES IN A MULTICENTER AND PROSPECTIVE COHORT STUDY – PROUDEST TRIAL

Brasília

2023

UNIVERSIDADE DE BRASÍLIA

HOSPITAL UNIVERSITÁRIO DE BRASÍLIA

HUB UNB EBSERH

PROGRAMA DE RESIDÊNCIA MÉDICA EM PATOLOGIA

DÉBORA LUIZA ALBANO FULGENCIO

STUDY BETWEEN THE EFFECTS OF SARS-COV-2 INFECTION AND PLACENTAL MORPHOLOGY FEATURES IN A MULTICENTER AND PROSPECTIVE COHORT STUDY – PROUDEST TRIAL

Projeto de Pesquisa apresentado à COREME – PRM – HUB/UnB como exame de Qualificação do relatório parcial do TCC do Programa de Residência Médica em Patologia.

Orientador (a): Prof. Dr. Gustavo Henrique Soares Takano

Brasília

2023

AGRADECIMENTOS

Em primeiro lugar, agradeço aos meus pais, Dária e Humberto, e minha irmã, Alice, que sempre me apoiaram a estudar e me deram bases para concretizar meus sonhos. Sem vocês, seria impossível realizar o sonho de estudar e me formar médica e agora finalizar essa residência médica, na especialidade que sempre sonhei.

Ao meu esposo, Rodrigo, que esteve ao meu lado desde o começo da graduação, principalmente na iniciação científica, e que me incentivou a ser uma profissional (e uma pessoa) melhor.

Aos meus colegas da Anatomia Patológica do HUB (UAPAT), por terem tornado esses três anos de residência em algo extremamente agradável.

Aos meus preceptores da residência, por todos os ensinamentos e paciência. Gostaria de agradecer principalmente ao Dr. Gustavo Takano e Dra. Aline Filha, por cumprirem muito bem o papel de me orientar, reparar meus erros, me instigarem a pesquisar e não permitirem que eu desistisse deste trabalho, mesmo após algumas frustrações.

Aos meus familiares, que estiveram do meu lado desde o princípio e que também acreditavam que meu sonho de ser patologista se concretizaria.

Aos meus amigos, por tantos momentos prazerosos e divertidos, que foram fundamentais para manter minha saúde mental antes e durante a residência.

Agradeço também à Dra. Lizandra, Dra. Lícia e Dr. Alberto, por confiarem a mim a confecção desse trabalho de pesquisa.

ABSTRACT

In December 2019, in Wuhan, China, several cases of pneumonia caused by a virus were reported by the World Health Organization (WHO), this disease was designated by the WHO as COVID-19. The local epidemic spread rapidly until it was classified as a global pandemic in March 2020. The infection presents itself clinically in different ways, and its most serious clinical presentation is the Severe Acute Respiratory Syndrome (SARS). Since pregnant women, based on other pandemics with respiratory conditions, are at high risk of complications and sequelae, when symptomatic, they were included in the most vulnerable group. In view of the damage that pregnant women and fetuses can suffer, the objective of this study was to evaluate the histopathological findings of the placentas of pregnant women who were or had been infected by SARS-CoV-2 in two hospitals in the Federal District. Between July 2020 and April 2021, 72 placentas of pregnant women who were infected with SARS-CoV-2, either previously or in an acute phase during labor, were analyzed. The placentas were sent to the Pathological Anatomy Unit immediately after delivery. Were examined fresh, and tissue samples fixed in formalin. The material was processed and the slides were stained with hematoxylin and eosin. All placentas were analyzed according to the Amsterdam Criteria (2016). A review of the patients' charts and a literature review were carried out. From the reviewed medical records of 72 pregnant women diagnosed with SARS-CoV-2, 79.2% had mild symptoms, 9.7% moderate, 6.9% severe, 2.8% severe with maternal deaths. It was found that 63.9% had comorbidities. Of the the anatomopathological study in the placentas, 17.8) accelerated villous maturation, and 2.6% decidual arteriopathy. In our study, there was no evidence of significant effect of COVID-19 on fetal growth, despite most placental weights being below the 10th percentile. The morphological and histopathological findings were discrete and nonspecific and could not be directly correlated with a possible vertical transmission of the virus. In conclusion, the infection with the SARS-COV-2 virus, COVID-19, may be associated with a higher prevalence of vascular lesions in the maternal placental stroma in this study.

Keywords: Pregnant, COVID-19, placenta, histopathology, vertical transmission, vascular lesions.

RESUMO

Em dezembro de 2019, em Wuhan, na China, vários casos de pneumonia causada por um vírus foram relatados pela Organização Mundial da Saúde (OMS), doença essa que foi designada pela OMS como COVID-19. A epidemia local se espalhou rapidamente até ser classificada como pandemia global em março de 2020. A infecção se apresenta clinicamente de diversas formas, sendo sua apresentação clínica mais grave a Síndrome Respiratória Aguda Grave (SARS). Como as gestantes, com base em outras pandemias com quadros respiratórios, apresentam alto risco de complicações e sequelas, quando sintomáticas, foram incluídas no grupo mais vulnerável. Tendo em vista os danos que gestantes e fetos podem sofrer, o objetivo deste estudo foi avaliar os achados histopatológicos das placentas de gestantes infectadas ou infectadas pelo SARS-CoV-2 em dois hospitais do Distrito Federal. Entre julho de 2020 e abril de 2021, foram analisadas 72 placentas de gestantes infectadas com SARS-CoV-2, previamente ou em fase aguda durante o trabalho de parto. As placentas foram encaminhadas para a Unidade de Anatomia Patológica imediatamente após o parto. Foram examinadas amostras de tecido a fresco e fixadas em formalina. O material foi processado e as lâminas coradas com hematoxilina e eosina. Todas as placentas foram analisadas de acordo com os Critérios de Amsterdam (2016). Foi realizada uma revisão dos prontuários dos pacientes e uma revisão da literatura. Dos prontuários médicos revisados de 72 gestantes diagnosticadas com SARS-CoV-2, 79,2% apresentaram sintomas leves, 9,7% moderados, 6,9% graves, 2,8% graves com óbitos maternos. Verificou-se que 63,9% apresentavam comorbidades. Do estudo anatomopatológico nas placentas, 17,8) maturação vilosa acelerada, e 2,6% arteriopatia decidual. Em nosso estudo, não houve evidência de efeito significativo do COVID-19 no crescimento fetal, apesar da maioria dos pesos placentários estar abaixo do 10º percentil. Os achados morfológicos e histopatológicos foram discretos e inespecíficos e não puderam ser diretamente correlacionados com uma possível transmissão vertical do vírus. Em conclusão, a infecção pelo vírus SARS-COV-2, COVID-19, pode estar associada a uma maior prevalência de lesões vasculares no estroma placentário materno neste estudo.

Palavras-chave: Gestantes, COVID-19, placenta, histopatológico, transmissão vertical e lesões vasculares.

SUMÁRIO

INTRODUCTION

In December 2019, in Wuhan, China, the World Health Organization (WHO) reported several cases of pneumonia caused by a previously unknown virus. Identified as RNA virus, belonging to the Coronaviridae family, genus *Coronavirus*, and it was called SARS-CoV-2 (severe acute respiratory syndrome Coronavirus 2), and the disease was designated by the WHO as COVID-19. The local epidemic spread rapidly until it was classified as a worldwide pandemic in March 2020. [1–8] Since then, more than 609 million confirmed cases and more than 6.5 million deaths have been reported globally.[9]

The transmission of SARS-CoV-2 includes inhalation of contaminated droplets/aerosols, and contact transmission via oral, nasal, and eye mucous. The infection presents itself clinically in several ways; most reported symptoms are cough, fever, asthenia, headache, dysgeusia and/or anosmia. Some more are cited as dermatitis, acute kidney failure, thromboembolic complications and gastrointestinal symptoms. Its severe symptoms include dyspnea, chest pain, mobility and/or sensitivity changes. The most serious clinical presentation is severe acute respiratory syndrome (SARS), a progressive and acute respiratory failure, which requires supplemental oxygen, sometimes mechanical ventilation, which can progress to death. [1,4,7,8,10–12]

As pregnant women, based on other pandemics with respiratory diseases (H1N1, MERS), had a high risk of complications and sequelae, when symptomatic, they were included in the most vulnerable group.[1,3,5,13–17] The risk factors for severe disease and death are similar in pregnancy to the general population, and include older age, obesity and medical comorbidities (such as hypertension and diabetes). [5,18–21]

The placenta is a maternal-fetal organ that constitutes the physical interface between the mother and the fetus, which is a provider of nutrients and components necessary for metabolic needs and immune systems for development and growth fetal.[22] Investigations of placentas of women infected with SARS-CoV-2 have suggested that there is a low likelihood of viral transplacental transmission. However, even at low rates, the potential effects of inflammatory and prothrombotic environments on placentas have been cited. [3–5,12,13,15–21,23–31] These data indicate that infections in fetal growth and development cannot be forwent. In view of the damage that pregnant women and fetuses can suffer, the objective of this study was to evaluate the histopathological findings of the placentas of pregnant women who were or were infected

with SARS-CoV-2, in a multicenter and cohort study, named PROUDEST (Pregnancy Outcomes and Child Development Effects of the SARS-CoV-2 Infection Study).

METHODS

Ethical Approvals

The PROUDEST study was approved by the Research Ethics Committee of the School of Medicine of the University of Brasilia (certified as 32359620.0.0000.5558). It was also registered in the Brazilian Registry of Clinical Trials (RBR65QXS2). All women participating were required to sign an informed consent form to join the project.

Study Design

The PROUDEST (Pregnancy Outcomes and Child Development Effects of the SARS-CoV-2 Infection Study) is a multicenter, longitudinal, prospective observational study that is conducted in two sequential stages: PREGNANT and BORN. The PREGNANT substudy followed up - until day 21 postpartum - pregnant women who were exposed to SARS-CoV-2 at any stage of gestation and compared them to a control group consisting of non-exposed pregnant women. The BORN substudy will follow the children of the women included in the preceding (PREGNANT) branch. These children will be assigned to two comparison groups (exposed and non-exposed) according to their mothers' in-pregnancy exposure status and will be followed by a multidisciplinary team of health professionals from birth to the age of 5 years.

Patients

The pregnant women included in the study were over 18 years old. Exposure to COVID-19 was defined as a first-time RT-PCR test, serology test, or rapid test that returns positive results during pregnancy and is confirmed by a second test. Non exposure to COVID-19 were defined as asymptomatic pregnant women with negative serology tests (immunoglobulin G [IgG] and immunoglobulin M [IgM] tests) and were called negative controls.

Placental Findings

Between June 2020 and May 2021, all placentas of patients which entered the study, after delivery, were sent fresh to the pathology laboratory for gross examination. They were examined according to a standardized protocol that consisted of the morphology and findings of the chord, membranes, maternal and fetal sides, measurement of placental dimensions and chord length, evaluation of the placental disc after trimming of the fetal membranes and umbilical cord, followed by serial sectioning through a 1.5-

cm interval and cut surface examination. Macroscopic alterations were recorded and sampled. The sides were fixed in 10% formalin for 48 to 72 hours. Representative samples of the umbilical cord (two sections), the membranes (one fetal membrane roll), and the chorionic plate (at least three, non-peripheral, sections including maternal and fetal surface) were submitted to paraffin embedding. All representative placental samples taken for microscopic evaluation were routinely processed, embedded, sectioned at 5 µm, and staining with hematoxylin and eosin.

Morphological and histological analyses were performed in all placentas using the Amsterdam Criteria, 2015, from the Amsterdam Placental Workshop Group Consensus Statement[32,33]. Assessed parameters included the analysis of placental vascular processes, such as maternal stromal-vascular lesion (developmental: superficial implantation, decidual arteriopathy, increased immature extravillous trophoblast; malperfusion: distal villous hypoplasia, accelerated villous maturation, villous infarcts; loss of integrity: abruptio placenta and marginal abruption), and as fetal stromal-vascular lesions (developmental: villous capillary lesions, delayed villous maturation, dysmorphic villi; malperfusion: obstructive lesions of umbilical cord, recent intramural fibrin in large fetoplacental vessels, chorionic plate or stem villous thrombi, avascular or karyorhectic villi; loss of integrity: large vessel rupture, small vessel rupture); of placental inflammatory-immune processes, as maternal inflammatory response (chorionitis, chorioamnionitis), as fetal inflammatory response (umbilical vasculitis, funisitis), features of chronic inflammation (chronic deciduous, villitis, intervillositis), and other processes (massive perivillous fibrin deposition, abnormal placental shape or umbilical insertion site, placenta accrete, meconium-associated changes and increased circulating nucleated red blood cells).

RESULTS

Of the 79 pregnant women in the study, 72 (91,1%) were exposed to SARS-CoV-2 and 7 (8,9%) were used as negative controls. 77 (97,4%) of them were younger than 40 years old. Of those who were exposed, 57 (79,2%) had mild symptoms, 7 (9,7%) moderate, 5 (6,9%) severe, 2 (2,8%) severe with maternal deaths (table 1).

Of the comorbidities found, 29 (36,7%) were healthy, 14 (17,7) had diabetes, 4 (5,1%) systemic arterial hypertension, 4 (5,1%) intrauterine growth restriction, 2 (2,5%) thyroid diseases, 3 (3,8%) anemia, 1 (1,3%) asthma, 1 (1,3%) Wolff-Parkinson-White Syndrome; 1 (1,3%) evolved with HELLP syndrome and 3 (3,8%) with preeclampsia. 9 (11,4%) of them had diabetes and associated systemic arterial hypertension, 8 (10,1%) had diabetes and other comorbidities and 2 (2,5%) had systemic arterial hypertension and others. Of those who were exposed to SARS-CoV-2, 46 (63,9%) had comorbidities (table 2).

For parity, 76 (96,2%) were singleton pregnancies and 3 (3,8%) were twins. In terms of childbirth, 11 (13,9%) were preterm, before 37 weeks of pregnancy were completed. Regarding the types of births, 55 (69,6%) were cesarians and 24 (30,4%) vaginals. For the babies, 50 (63,3%) were appropriate for gestational age, 16 (20,3%) small for gestational age and 13 (16,5%) large for gestational age. Of the complications in childbirth, 9 (11,4%) had acute fetal distress, 4 (5,1%) oligohydramnios, 5 (6,3%) hemorrhage, 1 (1,3%) acute fetal distress plus oligohydramnios, 2 (2,5%) evolved to maternal death and 1 (1,3%) to neonatal death (table 1).

Of the placental weights, 35 (44,3%) were between the 10th - 90th percentile for estimated gestational age, 43 (54,3%) were below the 10th percentile for estimated gestational age and 1 (1,3%) was above the 90th percentile for estimated gestational age. Of the relevant findings of the anatomopathological study in the placentas, 14 (17,8%) accelerated villous maturation, 1 (1,3%) delayed villous maturation, 1 (1,3%) intervillous thrombi, 2 (2,6%) decidual arteriopathy, 1 (1,3%) abnormal placental shape, 1 (1,3%) chorioamnionitis, 1 (1,3%) deciduitis, 1 (1,3%) chorangioma, 1 (1,3%) accelerated villous maturation (global), intervillous thrombi (segmental) and abnormal placental shape, 1 (1,3%) chorioamnionitis, subchorionitis, chronical vasculitis and focal villitis, 1 (1,3%) accelerated villous maturation (global), small foci of avascular villi and focal deciduitis, 1 (1,3%) accelerated villous maturation (global), chorioamnionitis, subchorionitis and deciduitis, 1 (1,3%) accelerated villous maturation (global), decidual

arteriopathy, villous infarcts (segmental), 1 (1,3%) villous infarcts (segmental) and chorionic plate thrombi, 1 (1,3%) accelerated villous maturation (global), decidual arteriopathy and karyorrhectic villi (table 2).

Table 1. Maternal and fetal epidemiological data. The ones highlighted in gray are the negative controls.

ID	Mother age	Gestacional week at delivery	Severity degree	Gestacional week at SARS-COV-2 diagnosis	Type of birth	Parity	Neonatal Weight	Pregnancy outcome
12	27	39 + 3	Mild	37	Cesarian	1	3816	Livebirth
150	40	40	Mild	25 + 3	Cesarian	1	3300	Livebirth
88	32	41	Mild	23 + 2	Cesarian	1	4418	Livebirth
141	33	37 + 4	Mild	18 + 2	Cesarian	1	2340	Livebirth
521	30	40 + 1	Mild	38 + 3	Cesarian	1	3680	Livebirth
505	39	29 + 5	Mild	14 + 6	Cesarian	2	1296/1010	Livebirth
532	37	39 + 1	Mild	39	Cesarian	1	3700	Livebirth
523	19	36 + 2	Moderate	35 + 2	Cesarian	1	3920	Livebirth
9	38	34 + 5	Severe	33 + 6	Cesarian	1	2810	Livebirth
530	35	30 + 5	Moderate	20 + 4	Cesarian	2	1316/1480	Livebirth
275	37	38 + 5	Mild	18 + 4	Cesarian	1	2860	Livebirth
46	31	39 + 1	Mild	32 + 5	Vaginal	1	3498	Livebirth
117	37	38	Mild	28	Cesarian	1	2120	Livebirth
116	29	41	Mild	31	Vaginal	1	3560	Livebirth
549	35	38 + 4	Mild	25 + 4	Cesarian	1	3120	Livebirth
118	42	37 + 6	Mild	22	Vaginal	1	3606	Livebirth
133	39	37 + 6	Mild	17 + 2	Cesarian	1	2792	Livebirth
114	39	41	Mild	20 + 3	Cesarian	1	3972	Livebirth
152	33	39 + 2	Mild	28 + 1	Vaginal	1	3022	Livebirth
20	33	40 + 2	Mild	33	Cesarian	1	3778	Livebirth
267	35	39 + 2	Mild	22 + 5	Vaginal	1	3350	Livebirth

271	38	37 + 4	Mild	9 + 1	Cesarian	1	3450	Livebirth
10	31	40 + 3	Severe (death)	40 + 3	Cesarian	1	4045	Livebirth
164	21	38 + 5	Mild	13 + 4	Vaginal	1	3022	Livebirth
1	32	39 + 6	Mild	36 + 5	Cesarian	1	3312	Livebirth
217	28	38 + 2	Moderate	10 + 2	Cesarian	1	3582	Livebirth
26	34	36 + 1	Mild	36	Cesarian	1	2350	Livebirth
169	38	39 + 1	Mild	21 + 4	Vaginal	1	3640	Livebirth
42	30	39 + 4	Mild	31 + 3	Cesarian	1	2932	Livebirth
584	22	38 + 3	Mild	28 + 3	Vaginal	1	3732	Livebirth
125	21	36 + 6	Mild	20 + 5	Vaginal	1	1922	Livebirth
238	36	39 + 1	Mild	9 + 3	Cesarian	1	3000	Livebirth
129	39	39 + 1	Mild	30 + 6	Cesarian	1	3708	Livebirth
240	26	38 + 6	Mild	4 + 5	Vaginal	1	2725	Livebirth
163	37	40 + 4	Mild	19 + 1	Vaginal	1	3182	Livebirth
527	37	36 + 5	Mild	9 + 4	Cesarian	1	1792	Livebirth
520	24	40 + 5	Mild	40 + 5	Cesarian	1	3755	Livebirth
130	19	41 + 1	Mild	30 + 1	Vaginal	1	3490	Livebirth
187	29	39	Mild	11 + 4	Vaginal	1	2700	Livebirth
506	28	40	Mild	6	Vaginal	1	2728	Livebirth
194	23	39 + 4	Moderate	15 + 5	Vaginal	1	3122	Livebirth
225	29	39 + 4	Mild	26 + 4	Cesarian	1	3086	Livebirth
16	25	39 + 1	Severe	39 + 1	Cesarian	1	4210	Livebirth
215	30	38 + 6	Mild	12	Cesarian	1	3178	Livebirth
518	26	24 + 1	Mild	7	Vaginal	1	620	Neonatal Death
555	37	39	Mild	36 + 1	Cesarian	1	3230	Livebirth
87	22	40 + 5	Mild	35 + 2	Cesarian	1	3234	Livebirth
536	29	37 + 2	Mild	32 + 1	Cesarian	1	2822	Livebirth
522	21	40 + 6	Moderate	40 + 6 TC	Cesarian	1	3315	Livebirth
550	35	34	Moderate	34	Cesarian	1	1515	Livebirth
546	37	40 + 3	Severe	40 + 3	Cesarian	1	3785	Livebirth

632	33	39	Mild	11 + 1	Vaginal	1	3300	Livebirth
528	25	37 + 4	Mild	21 + 2	Vaginal	1	3060	Livebirth
634	25	40	Mild	22 + 2	Vaginal	1	3642	Livebirth
537	23	35 + 2	Mild	12 + 5	Cesarian	1	1428	Livebirth
222	23	40 + 4	Moderate	11 + 3	Cesarian	1	3006	Livebirth
34	22	37 + 5	Mild	30 + 1	Cesarian	1	2314	Livebirth
23	-	37	Severe (death)	37	Cesarian	1	3300	Livebirth
35	27	38 + 5	Mild	35	Vaginal	1	3668	Livebirth
511	30	41	Mild	17 + 5	Vaginal	1	3504	Livebirth
504	38	38 + 4	Mild	7	Cesarian	1	3300	Livebirth
47	29	37 + 1	Mild	30 + 1	Cesarian	1	2982	Livebirth
637	33	38	Mild	3 + 2	Vaginal	1	2726	Livebirth
126	33	36	Mild	25	Cesarian	1	2090	Livebirth
85	52	37 + 2	Severe	34 + 4	Cesarian	2	2470/2466	Livebirth
638	33	37 + 4	Mild	27 + 2	Cesarian	1	3004	Livebirth
82	33	38 + 2	Mild	29 + 2	Cesarian	1	2924	Livebirth
6	36	40 + 1	Mild	36	Cesarian	1	3782	Livebirth
64	18	39 + 1	Mild	37 + 2	Vaginal	1	3426	Livebirth
202	21	37 + 1	Mild	4 + 2	Cesarian	1	2540	Livebirth
13	27	41	Severe	36 + 1	Cesarian	1	3915	Livebirth
517	35	39 + 4	-	-	Cesarian	1	3316	Livebirth
208	22	37	-	-	Cesarian	1	2110	Livebirth
278	31	39	-	-	Cesarian	1	3696	Livebirth
192	39	37	-	-	Cesarian	1	2946	Livebirth
230	34	38 + 6	-	-	Cesarian	1	3238	Livebirth
644	36	40 + 3	-	-	Cesarian	1	3220	Livebirth
529	32	39 + 2	-	-	Vaginal	1	3285	Livebirth

326

327

328

329 Table 2. Placental findings, maternal comorbidities and complications in childbirth. The
330 ones highlighted in gray are the negative controls.

331

ID	Mother age	Gestacional week at delivery	Maternal comorbidities	Complications in childbirth	Placental Weight	Relevant placental Findings
12	27	39 + 3	-	Acute fetal distress	585	No relevant findings
150	40	40	Obesity, gestational hypertension, thyroidopathy	Acute fetal distress	405	No relevant findings
88	32	41	Anemia	-	670	Chorioamnionitis
141	33	37 + 4	Gestational diabetes, intrauterine growth restriction	-	345	Accelerated villous maturation (global), small foci of avascular villi, focal deciduitis
521	30	40 + 1	-	-	475	No relevant findings
505	39	29 + 5	Diabetes	-	520	Accelerated villous maturation (global)
532	37	39 + 1	-	-	535	No relevant findings
523	19	36 + 2	-	Oligohydramnios	480	Accelerated villous maturation (partial)
9	38	34 + 5	-	-	410	Accelerated villous maturation (partial)
530	35	30 + 5	Anemia	Hemorrhage	205/240	Chorioamnionitis, subchorionitis, chronical vasculitis, focal villitis
275	37	38 + 5	Gestational diabetes, anemia	-	520	No relevant findings
46	31	39 + 1	Intrauterine growth restriction, anemia	Oligohydramnios	420	Chorangioma
117	37	38	Thyroidopathy	-	370	Accelerated villous maturation (global)

116	29	41	-	-	480	No relevant findings
549	35	38 + 4	-	-	360	No relevant findings
118	42	37 + 6	Gestational diabetes	-	425	No relevant findings
133	39	37 + 6	Diabetes, thyroidopathy, intrauterine growth restriction	-	385	No relevant findings
114	39	41	Thyroidopathy	-	520	No relevant findings
152	33	39 + 2	Gestational diabetes, thyroidopathy	-	400	No relevant findings
20	33	40 + 2	Gestational diabetes, obesity, anemia	-	475	No relevant findings
267	35	39 + 2	Gestational diabetes, thyroidopathy	-	575	Intervillous thrombi (segmental)
271	38	37 + 4	Gestational hypertension, gestational diabetes, thyroidopathy	-	375	Accelerated villous maturation (global)
10	31	40 + 3	-	Maternal death	575	No relevant findings
164	21	38 + 5	-	-	345	No relevant findings
1	32	39 + 6	Gestational diabetes	-	395	No relevant findings
217	28	38 + 2	-	Hemorrhage	410	No relevant findings
26	34	36 + 1	Preeclampsia, gestational diabetes, thyroidopathy	HELLP Syndrome	350	Accelerated villous maturation (global)

169	38	39 + 1	-	-	360	No relevant findings
42	30	39 + 4	Gestational diabetes	-	350	No relevant findings
584	22	38 + 3	Gestational hypertension	-	480	No relevant findings
125	21	36 + 6	Asthma, intrauterine growth restriction	-	330	No relevant findings
238	36	39 + 1	-	-	445	No relevant findings
129	39	39 + 1	Gestational diabetes	-	465	No relevant findings
240	26	38 + 6	Gestational diabetes	-	320	No relevant findings
163	37	40 + 4	Wolf-Parkinson-White syndrom	-	380	No relevant findings
527	37	36 + 5	Gestational hypertension, gestational diabetes, obesity, intrauterine growth restriction	-	305	Accelerated villous maturation (global), intervillous thrombi (segmental), abnormal placental shape
520	24	40 + 5	-	Acute fetal distress	645	No relevant findings
130	19	41 + 1	-	-	550	No relevant findings
187	29	39	Gestational hypertension, Gestational diabetes, thyroidopathy	-	330	No relevant findings
506	28	40	Intrauterine growth restriction	-	310	No relevant findings
194	23	39 + 4	Gestational diabetes	-	410	No relevant findings

225	29	39 + 4	-	Oligohydramnios	310	No relevant findings
16	25	39 + 1	-	-	610	Delayed villous maturation
215	30	38 + 6	-	Acute fetal distress, oligohydramnios	473	No relevant findings
518	26	24 + 1	-	Neonatal death	190	Accelerated villous maturation (global), Chorioamnionitis, subchorionitis, deciduitis
555	37	39	-	-	380	No relevant findings
87	22	40 + 5	Asthma	-	495	No relevant findings
536	29	37 + 2	Gestational hypertension, gestational diabetes	-	355	Accelerated villous maturation (partial)
522	21	40 + 6	-	-	555	Decidual arteriopathy
550	35	34	Gestational hypertension, preeclampsia, intrauterine growth restriction	-	200	Accelerated villous maturation (global), decidual arteriopathy, villous infarcts (segmental)
546	37	40 + 3	Gestational diabetes	-	575	No relevant findings
632	33	39	Gestational diabetes	Hemorrhage	405	No relevant findings
528	25	37 + 4	-	-	400	Accelerated villous maturation (global)
634	25	40	Gestational hypertension, gestational diabetes	-	505	Villous infarcts (segmental), chorionic plate thrombi
537	23	35 + 2	Intrauterine growth restriction	Acute fetal distress	180	Accelerated villous maturation (global)
222	23	40 + 4	-	Acute fetal distress	365	No relevant findings

34	22	37 + 5	Gestational hypertension	Acute fetal distress	255	Accelerated villous maturation (partial)
23	-	37	-	Acute fetal distress	760	Accelerated villous maturation (partial)
35	27	38 + 5	Gestational diabetes, sicke cell anemia traits	-	585	Abnormal placental shape
511	30	41	-	-	505	No relevant findings
504	38	38 + 4	-	-	405	No relevant findings
47	29	37 + 1	Gestational diabetes, intrauterine growth restriction	Oligohydramnios	510	No relevant findings
637	33	38	Gestational diabetes	-	500	No relevant findings
126	33	36	Gestational hypertension, preeclampsia, gestational diabetes	-	315	No relevant findings
85	52	37 + 2	Intrauterine growth restriction	Hemorrhage	530	No relevant findings
638	33	37 + 4	Intrauterine growth restriction	-	330	Decidual arteriopathy
82	33	38 + 2	Gestational diabetes, intrauterine growth restriction, anemia	-	300	No relevant findings
6	36	40 + 1	Gestational hypertension, gestational diabetes, obesity	-	570	No relevant findings

64	18	39 + 1	Gestational hypertension	Hemorrhage	505	Accelerated villous maturation (global), decidual arteriopathy, karyorrhectic villi
202	21	37 + 1	Intrauterine growth restriction	-	290	Accelerated villous maturation (global)
13	27	41	-	Acute fetal distress	545	Accelerated villous maturation (partial)
517	35	39 + 4	Gestational diabetes	-	442	No relevant findings
208	22	37	Gestational diabetes, gestational hypertension, intrauterine growth restriction	-	260	No relevant findings
278	31	39	-	-	490	No relevant findings
192	39	37	-	Acute fetal distress	480	No relevant findings
230	34	38 + 6	-	-	309	No relevant findings
644	36	40 + 3	Gestational diabetes	-	365	Deciduitis
529	32	39 + 2	Gestational diabetes, obesity	Hemorrhage	495	Accelerated villous maturation (global)

DISCUSSION/CONCLUSION

As SARS-CoV-2 is a virus, its etiological, pathophysiological and clinical findings are expected to be associated with a state of induced inflammation. This can manifest in different ways, being cited by histiocytic intervillositis, chronic villitis, systemic endothelial dysfunction and hypercoagulability conditions. These histological findings of inflammation, such as histiocytic intervillositis and chronic villitis, are reported to suggest a direct association with circulating viruses or a loss of host tolerance.[4,5,12,13,16,18–21,27–30] Systemic endothelial dysfunction is represented by a dysfunction of the renin-angiotensin system, vasoconstriction and oxidative stress by binding to angiotensin-converting enzyme 2 receptors. Coagulopathy is due to the increase of fibrinogen and D-dimer values, modest thrombocytopenia and enlargement of PT (Prothrombin Time) or aPTT (Partial Thromboplastin Time, Activated), and may be associated with propensity for thrombosis in fetal circulation, represented by intervillous thrombi. [2,13,31,34–36]

In our study, vascular alterations in the maternal stroma were the most prevalent, and accelerated villous maturation is present in several forms of placental insufficiency and is defined by the presence of small or short hypermature villi, usually accompanied by increased syncytial nodes and fibrin intervillous. Decidual arteriopathy is a lesion of the vascular wall, maternal face or membrane, with foamy macrophages. It may present with acute atherosis, fibrinoid necrosis, thrombosis, transmural hypertrophy, chronic perivasculitis, and persistence in endovascular trophoblasts. The maternal vascular malperfusion has as main risk factors maternal hypertensive disorders, such as gestational hypertension and preeclampsia, and has been associated with oligohydramnios, fetal growth restriction, preterm birth, and stillbirth.[21,30,32,37] In our study there was no evidence of significant effect of COVID-19 on fetal growth, despite most placental weights being below the 10th percentile. Most deliveries were caesarean, however, it cannot be confirmed whether there was a direct effect of SARS-CoV-2 infection.

Few studies that evaluate the transmission of SARS-CoV-2 from mother to child, which is correlated with the most severe cases of maternal condition and time of maternal infection. Some corroborate this transmission, due to the presence of angiotensin-converting enzyme 2 receptors found in the maternal-fetal interface, including placental trophoblasts and due to the presence of the virus RNA in amniotic fluid and its persistence in the neonate 24 h after birth. Others disagree, as no significant alterations are found in the morphological placental findings and few samples show

detection of the virus. [3,5,12,15,17,21,25,27,28,31] In our study, the morphological and histopathological findings were discrete and nonspecific, and could not be directly correlated with a possible vertical transmission of the virus.

This study has limitations, considering that the presence of the virus in placental tissue was not investigated, either by immunohistochemistry or direct research of viral RNA in the tissue molecular studies. However, pathological analysis was performed in a laboratory accredited and certified by the National Quality Control Incentive Program (PICQ). The pathologists involved in the study are experienced pathologists in the study, all the histopathological analysis and reports were issued following the diagnostic criteria of the Consensus Declaration of the Amsterdam Placental Workshop Group.

Therefore, infection with the SARS-COV-2 virus, COVID-19, may be associated with a higher prevalence of vascular lesions in the maternal placental stroma in this study. However, it is necessary to deepen the samples in order to confirm the reproducibility and definition of the clinical, obstetric and pediatric repercussions of these findings.

REFERENCES

[1] D.A. Schwartz, A. Dhaliwal, Infections in pregnancy with Covid-19 and other respiratory RNA virus diseases are rarely, if ever, transmitted to the fetus: Experiences with coronaviruses, parainfluenza, metapneumovirus respiratory syncytial virus, and influenza, Arch Pathol Lab Med. 144 (2020) 920–928. https://doi.org/10.5858/arpa.2020-0211-SA.

[2] I. Eljilany, A.N. Elzouki, D-dimer, fibrinogen, and il-6 in covid-19 patients with suspected venous thromboembolism: A narrative review, Vasc Health Risk Manag. 16 (2020) 455–462. https://doi.org/10.2147/VHRM.S280962.

[3] R. Sevilla-Montoya, A. Hidalgo-Bravo, G. Estrada-Gutiérrez, O. Villavicencio-Carrisoza, M. Leon-Juarez, I. Villegas-Mota, S. Espino-y-Sosa, I.E. Monroy-Muñoz, R.J. Martinez-Portilla, L.C. Poon, J.A. Cardona-Pérez, A.C. Helguera-Repetto, L.D. Gonzalez-García, C.D. Mora-Vargas, P. Mateu-Rogell, M. Rodriguez-Bosch, I. Coronado-Zarco, S. Acevedo-Gallegos, M. Aguinaga-Ríos, V.H. Ramirez-Santes, M.A. Ortiz-Ramirez, M. Valdes-Flores, M. Cortes-Bonilla, Evidence of possible SARS-CoV-2 vertical transmission according to World Health Organization criteria in asymptomatic pregnant women, Ultrasound in Obstetrics and Gynecology. 58 (2021) 900–908. https://doi.org/10.1002/uog.24787.

[4] T. Buckley, N. Singh, W. Shertz, Placental Pathology in COVID-19: Case Series in a Community Hospital Setting, Cureus. (2021). https://doi.org/10.7759/cureus.12522.

[5] A.M. Kotlyar, O. Grechukhina, A. Chen, S. Popkhadze, A. Grimshaw, O. Tal, H.S. Taylor, R. Tal, Vertical transmission of coronavirus disease 2019: a systematic review and meta-analysis, Am J Obstet Gynecol. 224 (2021) 35-53.e3. https://doi.org/10.1016/j.ajog.2020.07.049.

[6] R. Raschetti, A.J. Vivanti, C. Vauloup-Fellous, B. Loi, A. Benachi, D. De Luca, Synthesis and systematic review of reported neonatal SARS-CoV-2 infections, Nat Commun. 11 (2020). https://doi.org/10.1038/s41467-020-18982-9.

[7] H. Esakandari, M. Nabi-Afjadi, J. Fakkari-Afjadi, N. Farahmandian, S.M. Miresmaeili, E. Bahreini, A comprehensive review of COVID-19 characteristics, Biol Proced Online. 22 (2020). https://doi.org/10.1186/s12575-020-00128-2.

[8] N. Petrosillo, G. Viceconte, O. Ergonul, G. Ippolito, E. Petersen, COVID-19, SARS and MERS: are they closely related?, Clinical Microbiology and Infection. 26 (2020) 729–734. https://doi.org/10.1016/j.cmi.2020.03.026.

[9] World Health Organization (WHO), Global overview, 2022.

[10] M.Z. Tay, C.M. Poh, L. Rénia, P.A. MacAry, L.F.P. Ng, The trinity of COVID-19: immunity, inflammation and intervention, Nat Rev Immunol. 20 (2020) 363–374. https://doi.org/10.1038/s41577-020-0311-8.

[11] L.P. Sasaki, G.M. Fernandes, A.P. da Silva, F. Motta, C. Siracusa, I.P. Rabelo, A. dos S. Junior, P.S. França, P. Kurisky, R.M. Tristao, C. de Albuquerque, C. Gomes, L. Maria da Mota, A. Zaconeta, Cerebrospinal fluid analysis of pregnant women at early stages of COVID-19, Taiwan J Obstet Gynecol. 61 (2022) 672–674. https://doi.org/10.1016/j.tjog.2022.03.043.

[12] G. Giordano, C. Petrolini, E. Corradini, N. Campanini, S. Esposito, S. Perrone, COVID-19 in pregnancy: placental pathological patterns and effect on perinatal outcome in five cases, Diagn Pathol. 16 (2021) 88. https://doi.org/10.1186/s13000-021-01148-6.

[13] S.M. Glynn, Y.J. Yang, C. Thomas, R.L. Friedlander, K.A. Cagino, K.C. Matthews, L.E. Riley, R.N. Baergen, M. Prabhu, SARS-CoV-2 and Placental Pathology Malperfusion Patterns Are Dependent on Timing of Infection During Pregnancy, 2021. www.ajsp.com.

[14] G.M. Fernandes, F. Motta, L.M. Paravidine Sasaki, Â.P. Da Silva, A.M. Miranda, A.O. De Carvalho, A.P. Monteiro Gomides, A.A. De Sousa Munhoz Soares, A.D.C. Moreira Dos Santos, C. De Oliveira Alves, C.M. Gomes, C.C. De Siracusa, D.A. De Araújo, D.L. Mendonça-Silva, J.A. Lacerda De Jesus, K.N. Costa, M.E. Canellas De Castro, P.S. Kurizky, P.S. França, R. Tristão, Y.R. Pereira, L.C.G. De Castro, A.M. Zaconeta, C.P. De Albuquerque, L.M.H. Da Mota, Pregnancy outcomes and child development effects of SARS-CoV-2 infection (PROUDEST

Trial): Protocol for a multicenter, prospective cohort study, JMIR Res Protoc. 10 (2021). https://doi.org/10.2196/26477.

[15] M.C. Sharps, D.J.L. Hayes, S. Lee, Z. Zou, C.A. Brady, Y. Almoghrabi, A. Kerby, K.K. Tamber, C.J. Jones, K.M. Adams Waldorf, A.E.P. Heazell, A structured review of placental morphology and histopathological lesions associated with SARS-CoV-2 infection, Placenta. 101 (2020) 13–29. https://doi.org/10.1016/j.placenta.2020.08.018.

[16] F.M. Cribiù, G.A. Croci, A. Del Gobbo, T. Rizzuti, E. Iurlaro, M. Tondo, A. Viscardi, S. Bosari, S. Ferrero, Histological characterization of placenta in COVID19 pregnant women, European Journal of Obstetrics and Gynecology and Reproductive Biology. 252 (2020) 619–621. https://doi.org/10.1016/j.ejogrb.2020.06.041.

[17] L. Gao, J. Ren, L. Xu, X. Ke, L. Xiong, X. Tian, C. Fan, H. Yan, J. Yuan, Placental pathology of the third trimester pregnant women from COVID-19, Diagn Pathol. 16 (2021). https://doi.org/10.1186/s13000-021-01067-6.

[18] P.Z. Rebutini, A.C. Zanchettin, E.T.S. Stonoga, D.M.M. Prá, A.L.P. de Oliveira, F. da S. Dezidério, A.S. Fonseca, J.C.H. Dagostini, E.C. Hlatchuk, I.N. Furuie, J. da S. Longo, B.M. Cavalli, C.L.T. Dino, V.M. de C.H. Dias, A.P. Percicote, M.B. Nogueira, S.M. Raboni, N.S. de Carvalho, C. Machado-Souza, L. de Noronha, Association Between COVID-19 Pregnant Women Symptoms Severity and Placental Morphologic Features, Front Immunol. 12 (2021). https://doi.org/10.3389/fimmu.2021.685919.

[19] S. Stenton, J. Mcpartland, R. Shukla, K. Turner, T. Marton, B. Hargitai, A. Bamber, J. Pryce, C.L. Peres, N. Burguess, B. Wagner, B. Ciolka, W. Simmons, D. Hurrell, T. Sekar, C. Moldovan, C. Trayers, V. Bryant, L. Palm, M.C. Cohen, SARS-COV2 placentitis and pregnancy outcome: A multicentre experience during the Alpha and early Delta waves of coronavirus pandemic in England, EClinicalMedicine. 47 (2022) 101389. https://doi.org/10.1016/j.

[20] T. Menter, K.D. Mertz, S. Jiang, H. Chen, C. Monod, A. Tzankov, S. Waldvogel, S.M. Schulzke, I. Hösli, E. Bruder, Placental Pathology Findings during and after

SARS-CoV-2 Infection: Features of Villitis and Malperfusion, Pathobiology. 88 (2021) 69–77. https://doi.org/10.1159/000511324.

[21] C.R.V. Leal, R.A.M. Maciel, M.D. Corrêa Júnior, SARS-CoV-2 Infection and Placental Pathology, Revista Brasileira de Ginecologia e Obstetricia. 43 (2021) 474–479. https://doi.org/10.1055/s-0041-1730291.

[22] T. Guimarães, S.* Tiago, A. Bueno, C. Lima, A. Viana, P. Mota, V. Ribeiro Guedes, A importância do exame anatomopatológico da placenta em obstetrícia e neonatologia, n.d.

[23] D. Levitan, V. London, R.A. McLaren, J.D. Mann, K. Cheng, M. Silver, K.S. Balhotra, S. McCalla, K. Loukeris, Histologic and Immunohistochemical Evaluation of 65 Placentas from Women with Polymerase Chain Reaction–Proven Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection, Arch Pathol Lab Med. 145 (2021) 648–656. https://doi.org/10.5858/arpa.2020-0793-SA.

[24] H.C. Ezechukwu, J. Shi, M.A. Fowora, C.A. Diya, F. Elfaki, O.A. Adegboye, Fetoplacental transmission and placental response to SARS-CoV-2: Evidence from the literature, Front Med (Lausanne). 9 (2022). https://doi.org/10.3389/fmed.2022.962937.

[25] J.L. Hecht, B. Quade, V. Deshpande, M. Mino-Kenudson, D.T. Ting, N. Desai, B. Dygulska, T. Heyman, C. Salafia, D. Shen, S. V. Bates, D.J. Roberts, SARS-CoV-2 can infect the placenta and is not associated with specific placental histopathology: a series of 19 placentas from COVID-19-positive mothers, Modern Pathology. 33 (2020) 2092–2103. https://doi.org/10.1038/s41379-020-0639-4.

[26] C. Tasca, R.S. Rossi, S. Corti, G.M. Anelli, V. Savasi, F. Brunetti, M. Cardellicchio, E. Caselli, C. Tonello, P. Vergani, M. Nebuloni, I. Cetin, Placental pathology in COVID-19 affected pregnant women: A prospective case-control study, Placenta. 110 (2021) 9–15. https://doi.org/10.1016/j.placenta.2021.04.002.

[27] E.T. Patberg, T. Adams, P. Rekawek, S.A. Vahanian, M. Akerman, A. Hernandez, A. V. Rapkiewicz, L. Ragolia, G. Sicuranza, M.R. Chavez, A.M. Vintzileos, P. Khullar, Coronavirus disease 2019 infection and placental histopathology in

519 women delivering at term, Am J Obstet Gynecol. 224 (2021) 382.e1-382.e18.
520 https://doi.org/10.1016/j.ajog.2020.10.020.

521 [28] L. Bertero, F. Borella, G. Botta, A. Carosso, S. Cosma, M. Bovetti, M. Carosso, G.
522 Abbona, G. Collemi, M. Papotti, P. Cassoni, C. Benedetto, Placenta histopathology
523 in SARS-CoV-2 infection: analysis of a consecutive series and comparison with
524 control cohorts, (n.d.). https://doi.org/10.1007/s00428-021-03097-3/Published.

525 [29] D.A. Schwartz, E. Avvad-Portari, P. Babal, M. Baldewijns, M. Blomberg, A.
526 Bouachba, J. Camacho, S. Collardeau-Frachon, A. Colson, I. Dehaene, J.C.
527 Ferreres, B. Fitzgerald, M. Garrido-Pontnou, H. Gergis, B. Hargitai, A. Cecilia
528 Helguera-Repetto, S. Holmstrom, C.L. Irles, A. Leijonhfvud, S. Libbrecht, T.
529 Marton, N. McEntagart, J.T. Molina, R. Morotti, A. Nadal, A. Navarro, M.
530 Nelander, A. Oviedo, A.R.O. Otani, N. Papadogiannakis, A.C. Petersen, D.J.
531 Roberts, A.G. Saad, A. Sand, S. Schoenmakers, J.K. Sehn, P.R. Simpson, K.
532 Thomas, M. Yolotzin Valdespino-Vazquez, L.E. Van der Meeren, J. Van Dorpe,
533 R.M. Verdijk, J.C. Watkins, M. Zaigham, Placental Tissue Destruction and
534 Insufficiency From COVID-19 Causes Stillbirth and Neonatal Death From
535 Hypoxic-Ischemic Injury: A Study of 68 Cases With SARS-CoV-2 Placentitis
536 From 12 Countries, Arch Pathol Lab Med. 146 (2022) 660–676.
537 https://doi.org/10.5858/arpa.2022-0029-SA.

538 [30] E.D. Shanes, L.B. Mithal, S. Otero, H.A. Azad, E.S. Miller, J.A. Goldstein,
539 Placental Pathology in COVID-19, Am J Clin Pathol. 154 (2020) 23–32.
540 https://doi.org/10.1093/ajcp/aqaa089.

541 [31] E. Bloise, J. Zhang, J. Nakpu, H. Hamada, C.E. Dunk, S. Li, G.E. Imperio, L.
542 Nadeem, M. Kibschull, P. Lye, S.G. Matthews, S.J. Lye, Expression of severe
543 acute respiratory syndrome coronavirus 2 cell entry genes, angiotensin-converting
544 enzyme 2 and transmembrane protease serine 2, in the placenta across gestation
545 and at the maternal-fetal interface in pregnancies complicated by preterm birth or
546 preeclampsia, Am J Obstet Gynecol. 224 (2021) 298.e1-298.e8.
547 https://doi.org/10.1016/j.ajog.2020.08.055.

548 [32] T.Y. Khong, E.E. Mooney, I. Ariel, N.C.M. Balmus, T.K. Boyd, M.A. Brundler,
549 H. Derricott, M.J. Evans, O.M. Faye-Petersen, J.E. Gillan, A.E.P. Heazell, D.S.
550 Heller, S.M. Jacques, S. Keating, P. Kelehan, A. Maes, E.M. McKay, T.K.

Morgan, P.G.J. Nikkels, W.T. Parks, R.W. Redline, I. Scheimberg, M.H. Schoots, N.J. Sebire, A. Timmer, G. Turowski, J.P. Van Der Voorn, I. Van Lijnschoten, S.J. Gordijn, Sampling and definitions of placental lesions Amsterdam placental workshop group consensus statement, in: Arch Pathol Lab Med, College of American Pathologists, 2016: pp. 698–713. https://doi.org/10.5858/arpa.2015-0225-CC.

[33] R.W. Redline, Classification of placental lesions, Am J Obstet Gynecol. 213 (2015) S21–S28. https://doi.org/10.1016/j.ajog.2015.05.056.

[34] Evaluation of D-dimer and fibrinogen levels in COVID-19 patients, Annals of Clinical and Analytical Medicine. 13 (2022). https://doi.org/10.4328/acam.21126.

[35] J. Silva-Cardoso, E. Moreira, Renin-angiotensin-aldosterone system inhibitors and the COVID-19 epidemic, Revista Portuguesa de Cardiologia. 41 (2022) 831–832. https://doi.org/10.1016/j.repc.2022.08.006.

[36] M.C. Sousa, T.R. de-Santana, A.C.P. e Oliveira, F.S.B. Ferreira, F.D. Xavier, COVID-19 E TROMBOSE, Brasília Médica. 58 (2021). https://doi.org/10.5935/2236-5117.2021v58a69.

[37] O. Marie Faye-Petersen, D.S. Heller, V. V Joshi, Handbook of Placental Pathology Second Edition, n.d.