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Histopathological and clinical analysis of COVID-19-infected placentas

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Abstract

Background The impact of COVID-19 pandemic in pregnant patients is an important emerging topic. Evidence of transplacental transmission and typical histopathological alterations in the placenta are controversial in the literature.

Methods This was a prospective observational cohort multicenter study in which we selected 23 placentas of 21 patients that showed detection of SARS-CoV-2 RNA by RT-PCR in the placenta tissue and described both morphological and clinical characteristics. Immunohistochemistry was performed to localize the virus in the specimens.

Results Most of the patients were asymptomatic (61.9%) and preterm delivery was observed in 8 patients (34.7%). In relation to histopathological features, all the placentas showed evidence of maternal vascular malperfusion, as well as some degree of villitis with a high frequency of high grade placentitis (73.9%) and chronic and/or acute intervillositis (82.6%). We observed immunopositivity for SARS-CoV-2 antibody in 69.5% of the cases, notably in the syncytiotrophoblast.

Conclusions We reported histopathological features of placentas with viral detection in the tissue, thus providing evidence that SARS-CoV-2 can affect the placenta, although maternal and neonatal clinical outcome is usually mild.

Keywords Placenta, COVID-19, SARS-CoV-2, Histopathology, Clinical outcome

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for coronavirus disease 2019 (COVID-19), is a member of a family that includes viruses that cause diseases from a common cold to SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) (Jamieson et al. 2022; Fan et al. 2021). Similar to its predecessors, SARS-CoV-2 has been associated with poor outcomes during pregnancy, such as preterm birth, fetal death, preeclampsia, birth by emergency cesarian delivery and prolonged hospital admission after birth (Gurol-Urganci et al. 2021) Additionally, evidence of vertical transmission (antepartum and peripartum) is now recognized (Rebutini et al. 2021).

Transplacental SARS-CoV-2 transmission corresponds to almost 30% of neonatal infections, and the remaining transmission is associated with environmental exposure. Raschetti et al. observed that 55% of infected neonates developed COVID-19 (Raschetti et al. 2020). The detection of SARS-CoV-2 RNA in the placenta or membrane tissues was reported for the first time by Penfield et al. in August 2020, as supporting evidence of transplacental transmission. Viral load was detected in 3 out of 11 samples from COVID-19-positive women, with all presenting critical to severe disease at the time of delivery (Penfield et al. 2020). It is important to note that, at first, vertical transmission was controversial, since several studies failed to detect the virus in placental and membrane tissues, as well as amniotic fluid, vaginal secretion and breast milk (Schwartz et al. 2020a; Fan et al. 2021; Levitan et al. 2021). Studies using immunohistochemistry and in situ hybridization have provided consistent data showing viral localization in syncytiotrophoblast cells at the maternal–fetal interface and more rarely in Hofbauer cells and intervillous inflammatory cells (Patanè et al. 2020; Vivanti et al. 2020; Facchetti et al. 2020).

The histopathological analysis of the placenta is useful in providing valuable information regarding the mechanisms of maternal–fetal infection of a variety of pathological agents; characteristic morphological changes are associated with specific viruses, such as cytomegalovirus, Zika virus, dengue virus, and herpes (Rebutini et al. 2021; Bittencourt et al. 2002; Ribeiro et al. 2012; Benirschke et al. 2012). Although the determination of COVID-19-associated histopathological characteristics is controversial (Levitan et al. 2021; Suhren et al. 2022), some changes, such as fibrin deposition and intervillositis, have been frequently described in the placentas of COVID-19-infected women (Schwartz et al. 2020b; Mao et al. 2022).

This study aimed to describe the morphological characteristics of placentas with SARS-CoV-2 RNA detection and viral localization using immunohistochemistry in association with maternal clinical data.

Materials and methods

Study design and ethics

This was a prospective observational cohort multicenter study. The study was approved by the ethics committee from IRB IFF CAAE 34268020.5.0000.5269. Mothers signed informed consent forms. The authors followed all relevant guidelines, regulations, and ethics and safety protocols during all stages of the study's execution.

The present work is part of a major prospective cohort of pregnant women with laboratory-confirmed COVID-19 infection whose placental specimens were sent for histopathologic analysis. We selected a subcohort of SARS-CoV-2 RNA-detected specimens resulting in 23 placentas from 21 patients (2 women had twins with dichorionic diamniotic placentas). The majority of the women ($n=19$) were diagnosed near the site of delivery in the three participating institutions (Instituto Fernandes Figueira, Casa de Saúde Laranjeiras/Perinatal e Hospital Estadual Adão Pereira Nunes, Rio de Janeiro, Brazil).

Samples and histopathological evaluation

All placentas were examined according to a standardized protocol. Fresh specimens were grossly described with the measurements of placental dimensions, cord length, and weight followed by serial sectioning and cut surface examination. Specimens were fixed with 10% buffered formalin for 72 h and then sampled (at least 8 sections of the chorionic plate, including maternal and fetal surfaces, 2 sections of membranes and 3 sections of the umbilical cord). Sections were submitted to routine processing, paraffin embedding, sectioning at 5 µm, and staining with hematoxylin and eosin (H&E). Slides were microscopically assessed by two pathologists (including one experienced perinatal pathologist) and evaluated according to the Amsterdam Placental Workshop Group Consensus Statement.

Morphological parameters included characteristics of: maternal vascular malperfusion (MVM), such as villous infarction, intervillous thrombosis, increased fibrin deposition with or without necrosis of the trophoblast, decidual vasculopathy with thrombosis, acute atherosis, vascular fibrinoid necrosis; features of fetal vascular malperfusion (FVM), such as fetal vascular thrombosis, avascular villi, villous stromal-vascular karyorrhexis, vascular ectasias and congestion, fibromuscular hypertrophy of fetal blood vessels and subintimal fibrin deposition, features of inflammatory response, including villitis, intervillositis, deciduitis, chorionitis, subchorionitis and

umbilical vasculitis/funisitis and, finally, chorangiosis and delayed villous maturation.

Clinical information

Clinical and laboratory data were retrieved from medical records and included maternal age, comorbidities, gestational age at delivery, gestational age at the moment of COVID-19 infection, presence of typical symptoms of COVID-19, disease severity, APGAR score, neonatal outcome and serological status (available for 12 newborns).

Immunohistochemistry

The paraffin-embedded tissues (4 μm thick) were incubated for 90 min at 60 °C, deparaffinized in xylene, and rehydrated with ethanol. Antigen retrieval was performed by heating the tissue in the presence of citrate buffer (pH 6.0). Tissue sections were then incubated with 3% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase and rinsed in Tris–HCl (pH 7.4). Slides were then rinsed in Protein Blocker solution (ScyTek, Logan, Utah, USA) for 10 min to reduce nonspecific binding. Samples were incubated with primary antibody (hyperimmune globulins against the spike glycoprotein of SARS-CoV-2, produced in mice, FIOCRUZ/RJ) at 4 °C overnight (dilution 1:500). Subsequent incubations were performed with the Two-Step Polymer Immunohistoprobe Plus (Redwood, California, USA) amplifier for mouse and amplifier for rabbit IgG for 15 min and HRP polymer detector at room temperature for 15 min. Samples were exposed to diaminobenzidine (ScyTek, Logan, Utah, United States), and Mayer's hematoxylin (Dako, Palo Alto, California, USA) was used for counterstaining. Sections were analyzed with an Olympus BX 53 microscope, and images were acquired using a coupled Olympus DP72 camera.

RT–PCR

To confirm the presence of viral particles in tissue by real-time PCR, placental fragments were collected in 1 mL of TRIzol Reagent and placed in liquid nitrogen until tissue processing. Tissue samples were then subjected to 4 cycles of standardized mechanical dissociation (6 m/s, 30 s) using the L-Beader system (Loccus, Cotia, São Paulo, Brazil). After that, the samples were centrifuged (460xG, 2 min), and the supernatant was collected. The placenta macerate in TRIzol was applied to the BDmax (BD, Franklin Lakes, New Jersey, USA) semiautomated total nucleic acid extraction and One-Step Real Time RT–PCR system. Formalin-fixed paraffin-embedded (FFPE) tissue samples were submitted to microtomy of 50μm total slices per case. The sections were deparaffinized in xylene, treated in absolute alcohol and conditioned in 1 mL of TRIzol Reagent. Then, the samples were subjected to incubation at 80 °C, tissue dissociation by L-Beader Loccus and centrifugation as previously described, and 50 μl of the macerated supernatant was applied to the BDmax system. Briefly, the BDmax system used the magnetic method of total DNA and RNA extraction in a standardized way, resulting in a final volume of 25 μL. Immediately after full extraction, 12.5μl of the extracted material was used by the system to elute the lyophilized BDmax master mix. This mixture was added to 12.5μl of 2×concentrated primer and probe solution, finalizing 25μl of solution for PCR prepared by the BDmax system. Finally, the equipment performed the application of 12.5μl of the PCR solution in BDmax microplates, and each well used was sealed by heating. After that, the equipment started reverse transcription followed by real-time PCR for SARS-CoV-2 Gene E according to the amplification conditions of the Berlin protocol (Corman et al. 2020) using the following sequences of primers and probes: E_Sarbeco_F ACAGGTACGTTAATAGTTAATAGCGT (400 nM), E_Sarbeco_R ATATTGCAGCAGTACGCACACA (400 nM) and E_Sarbeco_P1 FAM-ACACTAGCCATCCTT ACTGCGCTTCG-BBQ (200 nM). In addition, patented synthetic RNA was also used as an internal extraction and amplification control in all reactions performed in the BDmax system.

Statistical analysis

Descriptive statistics were performed using frequency and mean, when applicable.

Results

Relevant clinical data are summarized in Table 1. COVID-19 was frequently diagnosed during the third trimester due to routine testing at maternity admission. Importantly, most of the women did not present typical COVID-19 symptoms (57.1%). Preterm delivery was observed in 8 newborns (34.7%), in which 5 newborns had worse outcomes, with 4 deaths (including one twin) and 1 with severe respiratory disease (hyaline membrane disease). In relation to serological status, when available, all newborns, except for one, were COVID-19 IgA negative. Interestingly, two preterm newborns from a twin pregnancy showed SARS-CoV-2 viral detection by PCR in nasal swabs. Regarding maternal preexisting disease in addition to COVID-19, preeclampsia was found in 28.5% of the mothers.

In relation to histopathological features (Table 2), all the placentas showed evidence of MVM, especially intervillous infarcts, increased fibrin villous deposition and decidual vasculopathy. It is relevant to note that only 4 patients had preeclampsia. In addition, all of the placentas showed some features of FVM. Additionally, all of

Table 1. Patient's clinical characteristics

Case	Maternal age	Maternal comorbities	Gestational age at delivery	Gestational age at diagnoses	COVID-19 symptoms	APGAR (1min/5min)	Neonatal outcome	Fetal sorological status	Single or twin pregnancy
1	27	athsma, ppd*	37+4	36+6	no	(7/8)	no relevant findings	IgA negative/IgG not available	single
2	32	absence	38	32+6	no	(9/10)	no relevant findings	IgA negative/IgG positive	single
3	22	hipothyroidism, pre-eclampsia	38+1	18+6	no	(9/10)	no relevant findings	IgA negative/IgG negative	single
4	37	hipothyroidism, diabetes	36+2	32	no	(9/9)	preterm delivery	IgA negative/IgG positive	single
5	41	absence	41+3	19+4	no	(8/9)	bradicardia	IgA negative/IgG negative	single
6	37	absence	37	35+4	no	(9/10)	no relevant findings	IgA negative/IgG negative	single
7	25	renal litiasis	40+4	40	yes/mild**	(8/9)	no relevant findings	not available	single
8	36	absence	40	30+4	no	(10/10)	no relevant findings	IgA negative/IgG negative	single
9	34	thrombophilia	38	34+4	no	(9/10)	no relevant findings	IgA positive/IgG positive	single
10	40	pre-eclampsia	35	32+6	no	(10/10)	preterm delivery	IgA negative/IgG positive	single
11	37	pre-eclampsia, diabetes, hipothyroidism	37	36+6	no	(9/10)	no relevant findings	not available	single
12	38	absence	38	38	yes/mild**	fetal death		not available	single
13	42	diabetes	37+1	37+1	no	(9/9)	no relevant findings	not available	single
14	35	HIV	37	37	no	(7/9)	no relevant findings	not available	single
15	33	absence	39+2	38+2	yes/mild**	(9/10)	no relevant findings	not available	single
16	26	pre-eclampsia	37	37	yes/mild**	(9/9)	no relevant findings	not available	single
17	37	pre-eclampsia	37	35+4	yes/mild**	(9/9)	no relevant findings	not available	single
18	25	absence	30	30	yes/severe***	preterm delivery+fetal death		not available	single
19	25	absence	32	32	yes/severe***	preterm delivery+fetal death		not available	single
20	42	pre-eclampsia	32+6	31+6	yes/severe***	not available	hialin membrane disease + fetal	not available	twin
21	40	absence	34+2	34	yes/mild**	(8/9)	preterm delivery	not available	twin

Abbreviation: **ppd* premature placental detachment. **Mild symptoms included cough, sinusitis, myalgia and fever. ***Severe clinical course included dyspnea, intensive care unit hospitalization and oxygen dependence.

Table 2 Placental histopathological characteristics

	Morphological Features	**n**	**%**
Placental weight	Adequate for gestational age	11	47.8
	Small for gestational age	11	47.8
	Large for gestational age	1	4.4
MVM	Decidual vasculopathy	16	69.5
	Intervillous thrombosis	16	69.5
	Infarcts	22	95.6
	Fibrin deposition increase	19	82.6
FVM	Fetal vascular thrombosis and Subintimal fibrin deposition	17	73,9
	Avascular villi and *Stromal-vascular karyorexis*	20	86,9
Inflammatory changes	Chronic and/or acute intervillositis	19	82.6
	High grade placentitis	17	73.9
	Chorionitis and/or subchorionitis	13	56.5
UC	Funicular vascular thrombi	8	34.7
Imunohistochemistry for Sars-CoV-2	Positive	16	69.5
	Negative	8	30.5

Abbreviations: *MVM* Maternal vascular malperfusion, *FVM* Fetal vascular malperfusion, *UC* Umbilical cord. Placenta weight mean 348.14g (SD122,27)

the specimens showed some degree of villitis with a high frequency of high grade placentitis (73.9%) and chronic and/or acute intervillositis (82.6%), showed in Fig. 1. Very few alterations were found at the umbilical cord. Interestingly, one of the twin pregnancies showed discordant findings between the placentas with increased inflammatory alterations in one compared with the other. The twin case that resulted in fetal death showed an equal degree of inflammatory and vascular response in both placentas.

All samples had SARS-CoV-2 RNA detected by qPCR as an inclusion criterion; however, we wanted to demonstrate viral localization in the placental disc by immunohistochemistry. We observed immunopositivity in 69.5% of the cases, notably in the syncytiotrophoblast, with few positive cells in the villous stroma (Fig. 1).

Discussion

This study is part of a large prospective cohort of pregnant COVID-19-infected women who delivered in 2020 and 2021. In this cohort, we aimed to analyze placentas that had SARS-CoV-2 RNA detected in placental tissue as evidence of transplacental transmission. Additionally, we performed immunohistochemistry to localize viral particles in the samples. Schwartz proposed that transplacental transmission criteria should include the use of immunohistochemistry and RNA in situ hybridization/RNAscope to identify the SARS-CoV-2 virus in cells on the fetal side of the placenta from maternal/neonatal dyads that test positive for COVID-19 (Schwartz et al. 2020a).

Several studies have reported viral particle localization in the syncytiotrophoblast membrane (Patanè et al. 2020; Facchetti et al. 2020; Rakheja et al. 2021). The molecular mechanisms by which the virus might reach the maternal–fetal interface are still unclear. Importantly, the angiotensin-converting-enzyme 2 (ACE2) receptor and the serine protease TMPRSS2 are widely associated with cell entry and dissemination of SARS-CoV-2 (Shang et al. 2020; Wang et al. 2020; Hoffmann et al. 2020). Studies have identified both ACE receptor and TMPRSS2 in the human placenta; however, some results are conflicting. Taglauer and colaborators showed that both ACE2 and SARS-CoV-2 spike glycoprotein consistently localized within the syncytiotrophoblast membrane, although the expression of ACE2 was downregulated in comparison with the control group. ACE2 expression might decrease during pregnancy, and it seems that ACE2 is downregulated in the third trimester compared with the first

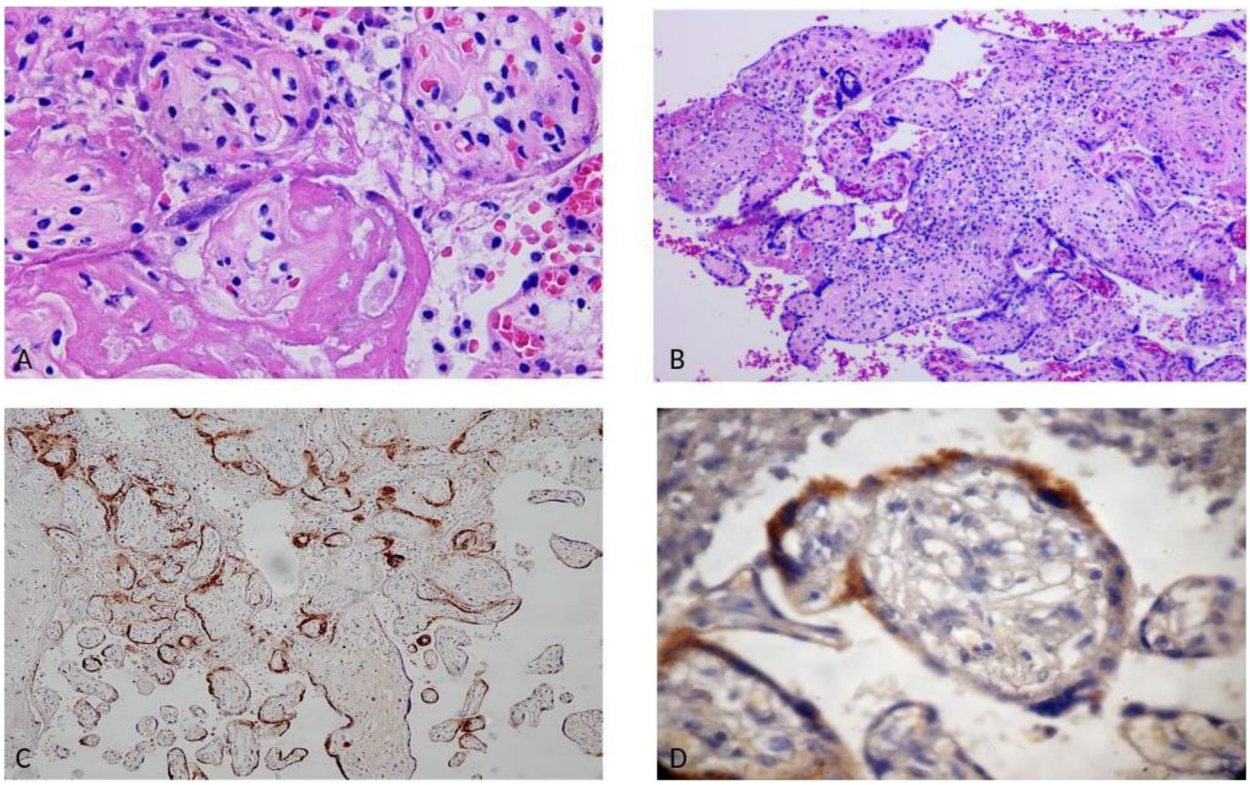

Fig. 1 Microscopic features and immunohistochemistry for SARS-CoV-2 in examined placentas. **A** Fibrin perivillous deposition with chronic and acute villous deposition (10x). **B** Proliferative villitis (4x). **C** Immunohistochemistry for SARS-CoV-2 showing diffuse positivity in the syncytiotrophoblast (10x). **D** High power field showing positivity in the syncytiotrophoblast (40x)

trimester placenta (Taglauer et al. 2020). Pique-Regi and others found consistent data, showing very low levels of ACE2 and TMPRSS2 in the human placenta, including in samples of chorionic membranes and first trimester placenta (Pique-Regi et al. 2020). These findings are evidence that does not support direct vertical transmission, although some authors suggest that other proteins might be involved in SARS-CoV-2 cell entry and spread during pregnancy (Pique-Regi et al. 2020; Gordon et al. 2020). Another hypothesis is that since both SARS-CoV-2 spike protein and ACE2 are expressed in fetal organs, transmission to the fetus might occur through amniotic fluid (Beesley et al. 2021).

A recent meta-analysis proposed that there are no specific alterations in infected women's placentas, since the rates of inflammatory and maternal vascular malperfusion alterations in placentas of COVID-19-infected mothers are similar to those expected in non-COVID-19 placentas in the literature (Suhren et al. 2022). Although there is a lack of studies using control groups, to our knowledge, this meta-analysis failed to show statistical evidence to compare the outcomes between the studies. Some histological patterns typically associated with COVID-19 infection are MVM and FVM changes, massive perivillous fibrin deposition and inflammatory patterns, including acute and chronic chorioamnionitis, chronic histiocytic intervillositis and villitis of unknown etiology (VUE) pattern (Wong et al. 2022). Abnormal placental findings have been associated with stillbirths and low Apgar scores (<7) in the first and fifth minute (Joshi et al. 2022).

In our study, frequent alterations of MVM were infarct, massive perivillous fibrin deposition, intervillous thrombosis and decidual vasculopathy with decidual thrombosis. The spectrum of MVM alterations varies in the literature. Shanes et al. found that acute atherosis and fibrinoid necrosis, as well as mural hypertrophy, were significantly higher in COVID-19 cases than in a control group (Shanes et al. 2020). Taglauer et al. reported that 14 of the 15 (93%) third trimester placentas presented at least a feature of MVM, with infarcts and increased fibrin deposition being the most frequently observed, compared to healthy controls where MVM was observed in only 30% (3/10) of cases (Taglauer et al. 2020). MVM was also the most frequent morphological alteration in the study of Joshi and others (Joshi et al. 2022). In contrast, Hecht et al. showed a low frequency of MVM in their cohorts; however, COVID-19-exposed cases revealed decidual vasculopathy (Hecht et al. 2020). Zhang et al. reported frequent massive perivillous fibrin deposition; however, this finding was not statistically significant when compared to the control group (Zhang et al. 2020). In particular, an increased number of syncytial knots and maternal vascular thrombosis were few characteristics that were significantly more common in the COVID-19 cases than in the control group in the study of Wong et al. In this study, the authors propose that maternal hypoxia secondary to COVID-19 lung infection might lead to uterine malperfusion and consequently hypoxic-ischemic injury in the placenta (Wong et al. 2022).

FVM alterations are frequently reported in COVID-19-affected placentas with a variable frequency (from 8% in one study to 75% of cases in another study) (Joshi et al. 2022; Patberg et al. 2021). When compared in groups selected by aggressiveness of the maternal symptoms, FVM features did not show statistical significance in multiple studies (Patberg et al. 2021; Arcos-Junior et al. 2022). It seems that the time of infection influences the rates of these alterations, as shown by Glynn and others who demonstrated a significantly higher frequency of FVM changes between patients infected with SARS-CoV-2 within 14 days of delivery admission and women infected prior to 14 days before delivery admission (Glynn et al. 2021). Our study was mainly conducted with pregnant women tested at delivery admission or very close to delivery (less than 14 days), except for 3 patients. All of the women in our cohort showed some features of FVM, with stromal-vascular karyorrhexis being the most common finding (86.9%), followed by fibromuscular hypertrophy of fetal vessels (82.6%), fetal vascular thrombosis (65.3%) and avascular villi (56.5%). The last two were reported in 16% of the cases and showed significant differences when compared to a control group in a cohort with 50 cases (Al-Rawaf et al. 2022). FVM in the context of COVID-19 may represent a pregnancy-specific consequence of coagulopathy, which might result from a combination of the inflammatory response to the virus and microvascular injury from direct viral damage within endothelial cells (Glynn et al. 2021).

Placentitis is a well-studied histopathological alteration, since it is an expected finding in variable infectious diseases that might affect the placenta. Viruses associated with TORCH (*Treponema pallidum*, *Toxoplasma gondii*, rubella virus, cytomegalovirus and herpes virus) pathogens are associated with chronic villitis in placentas (Bittencourt et al. 2002). Observed in Ireland for the first time in 2020, the recognized pattern called 'SARS-CoV-2 placentitis' is defined by chronic histiocytic intervillositis associated with massive perivillous fibrin deposition. This was first described by Linehan et al. (Linehan et al. 2021), who also showed the presence of the virus within syncytiotrophoblasts. SARS-CoV-2 placentitis has also been proposed by other groups and might be linked to an increased risk of vertical transmission (Schwartz et al. 2020b; Schwarts et al. 2020c; Stenton et al. 2022). Stenton and others suggest that chronic histiocytic intervillositis

might be a first response to the infection and fibrin deposition as a secondary event with or without trophoblast necrosis (Stenton et al. 2022). Also, this pattern might be linked to SARS-CoV-2 intrauterine transmittability and hypoxia (Mao et al. 2022). In our study, we observed a significant frequency of both chronic intervillositis and diffuse fibrin deposition. Another pattern of inflammatory alterations commonly studied in COVID-19-infected placentas was chronic villitis/villitis of unknown etiology-like (VUE). Patberg et al. observed a frequency of chronic villitis/VUE three times higher in COVID-19 cases than expected in normal placentas (Patberg et al. 2021). In a study by Wong and others, chronic villitis was significantly increased in COVID-19-infected placentas compared to controls (Wong et al. 2022). Similar results were reported by Redline et al., especially when term (> 37 weeks) placentas were selected (Redline et al. 2022). The mechanisms causing VUE have yet to be described; however, it is implied to a certain extent that an increased immune response following the cytokine storm might lead to this alteration. We also observed an acute inflammatory pattern, such as proliferative villitis and acute and chronic intervillositis, which are not commonly reported in the literature.

We showed a high frequency of umbilical cord changes in our study. Few studies have reported umbilical cord histopathology; however, our results are similar to these works, highlighting umbilical cord thrombosis (Al-Rawaf et al. 2022).

Although many groups have shown that placental histopathology does not seem to correlate with symptoms or even disease aggressiveness (Arcos-Junior et al. 2022; Resta et al. 2022; Stenton et al. 2022; Gulersen et al. 2020) common maternal and perinatal outcomes that have been associated with pathological alterations include stillbirths, Apgar score < 7 at the first and fifth minute, preterm delivery, and neonatal and maternal death (Wong et al. 2022; Joshi et al. 2022; Schwartz 2020d). In relation to fetal outcome, Apgar scores were > 7 in all live birth cases, and the majority of neonates did not show any clinical complications. Our cohort showed that the majority of the mothers had mild symptoms, and the placentas exhibited frequent inflammatory and vascular alterations, as described. Asymptomatic or oligosymptomatic women seem to show pathological features in the placenta similar to those of women who experience severe disease (Stenton et al. 2022; Schwartz 2020d; Daclin et al. 2022). We reported four cases of fetal death, and only in one case did the mother have a severe COVID-19 clinical history.

In regard to vertical transmission, although our inclusion criterion was evidence of SARS-CoV-2 RNA in placental tissue as an evidence of transplacental transmission, with exclusion of fetal death cases, our cohort did not show a high frequency of neonatal adverse outcomes. One of the neonates showed IgA positivity, and two neonates (twins) had RT–PCR positivity for SARS-CoV-2; however, no COVID-19 symptoms were observed in the infants.

Interestingly, in our study, one of the pairs of twins (with one stillbirth) exhibited significant differences between the placentas regarding the inflammatory alterations, showing exuberant villitis in one of the placentae (stillbirth correspondent). Moriarty and others reported a case with similar findings (Moriarty et al. 2022).

We included unvaccinated women since the period of the study included 2020 and 2021, when vaccination was not available in Brazil. Schwartz supports that placentas with abnormal findings, including the SARS-CoV-2 placentitis pattern with massive fibrin deposition and trophoblastic necrosis, are probably related to maternal viremia and can lead to stillbirth and neonatal death via placental malperfusion and insufficiency. Vaccination is highly effective in reducing the pathogenic effects resulting from viremia and thus is an important strategy to prevent fatal neonatal outcomes (Schwartz 2022).

One strength of this study was the use of both immunohistochemistry (IHC) with anti-spike glycoprotein of SARS-CoV-2 as well as RT–PCR to detect viral RNA in the tissue. IHC has been used by multiple authors with well demonstrated effectiveness in detecting and localizing viral particles in the placenta. The primary antibody used in our study was produced in house (Fundação Oswaldo Cruz/Fiocruz) from immunized mice with trimeric spike glycoprotein (Residues 1–1208) in the prefusion conformation for production of hyperimmune globulins against SARS-CoV-2. Since it was not a fully commercially available antibody at the time of our study, the in house produced antibody was extensively tested.

Frequency of viral antigen expression in the placenta varies in the literature. Some studies report a high frequency of positivity in IHC, which might be due to the widespread presence of Fc receptors in the human placenta (known to bind different affinity antibodies) leading to nonspecific staining (Rakheja et al. 2021; Schwartz et al. 2020c); however, we used molecular validation in order to reduce this effect. In contrast, there are a few studies showing low frequency of IHC positivity in the placenta even with viral demonstration by other methods (PCR, in situ hybridization or electron microscopy) (Reagan-Steiner et al. 2022; Hecht et al. 2020). Some authors attribute placental PCR positive cases with negativity by IHC to maternal viremia, however this is controversial (Reagan-Steiner et al. 2022).

Our results showed higher frequencies of histopathological alterations than those reported in the literature,

which might be due to our extensive sample of placentas (at least 8 FFPE blocks). Since there is evidence that placental alterations might be focal (Gulersen et al. 2020) our large sample method might have highlighted cases that would have been considered normal if it had not been properly sampled.

In the major cohort of placentas received in our institution from mothers infected with COVID-19, we did not observe a difference in relation to the frequency of histopathological changes when compared to placentas from COVID-19-infected mothers but with no evidence of tissue presence of the SARS-CoV-2 virus (from molecular or IHC testing) (data not published).

We reported histopathological features of placentas with viral detection in the tissue, thus providing evidence that SARS-CoV-2 can affect the placenta and might sporadically infect the infant. We also described clinical characteristics as well as maternal and neonatal outcomes. A limitation of the study is the lack of more detailed clinical information and fetal serological or PCR tests. The study of the placenta will continue to be important to provide useful information on COVID-19 and other recently emerged infections in the context of pregnancy.

Abbreviations

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus disease 2019
MERS	Middle East respiratory syndrome
PCR	Polymerase chain reaction
RT–PCR	Real-time polymerase chain reaction
FFPE	Formalin-fixed paraffin-embedded
PPD	Premature placental detachment
MVM	Maternal vascular malperfusion
FVM	Fetal vascular malperfusion
UC	Umbilical chord
SD	Standard deviation
ACE2	Angiotensin-converting-enzyme 2
VUE	Villitis of unknown etiology

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Authors' contributions
ACMN, EAP, TCC, PB, RA, ZV, MEM conceptualized and designed the study. ACMN, EAP, MMB, TCC, NS, ESC, DNL collected the data for the study. All authors contributed analysis and interpretation of data and to writing and/or editing of the manuscript and have seen the final version of the manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This work was approved by the ethics comitee of Fernandes Figueira Institute (CAAE 30598020.0.0000.526).

Consent for publication
Not applicable.

Competing interests
The authors declare no conflicts of interest.

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