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Pandemic stress and SARS-CoV-2 infection are associated with pathological changes at the maternal-fetal interface

Marie-Eve Brien [a,b], Dorothée Bouron-Dal Soglio [a,c], Solenn Dal Soglio [a], Camille Couture [a,b], Isabelle Boucoiran [a,d,e], Youssef Nasr [f], Kate Widdows [g], Megan C. Sharps [g], Dina El Demellawy [f,h], Alexander EP Heazell [g], Didier Menzies [i,j], Sylvie Girard [a,b,c,d,*]

[a] CHU Sainte-Justine Research Center, Montreal, Quebec, Canada
[b] Department of Microbiology, Infectiology and Immunology, Université de Montréal, Montreal, Quebec, Canada
[c] Department of Pathology and Cell Biology, Université de Montréal, Montreal, Quebec, Canada
[d] Department of Obstetrics and Gynecology, Université de Montréal, Montreal, Quebec, Canada
[e] School of Public Health, Université de Montreal, Montréal, Quebec, Canada
[f] University of Ottawa, Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, Canada
[g] Maternal and Fetal Health Research Centre, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom
[h] The Children's Hospital of Eastern Ontario, Department of Pathology and Laboratory Medicine, Ottawa, Canada
[i] Departement D'Anatomie et Cytologie, CHR de Metz-Thionville, France
[j] Departement of Pathology of Laboratoire National de Santé, Luxembourg

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ABSTRACT

Introduction: The reported effects of SARS-CoV-2 on pregnancy outcomes are conflicting; studies frequently overlook the placenta, which is critical for the health of the mother and infant(s). This study aimed to determine the effect of pandemic stress ± SARS CoV-2 infection on placental histopathology.
Methods: Women were recruited in Canada (n = 69); France (n = 21) or in the UK (n = 25), between March and October 2020. Historic controls (N = 20) were also included. Placenta and fetal membrane samples were collected rapidly after delivery and were fixed and stained for histopathological analysis. Maternal demographical data and obstetric outcomes were recorded.
Results: Over 80% of the placentas from SARS-CoV-2+ pregnancies had histopathological abnormalities: predominantly structural (71–86%) or inflammatory (9–22%), depending on geographical location. Excessive fibrin was seen in all sites, whereas deciduitis (Canada), calcifications (UK), agglutinations and chorangiosis (France) predominated in different locations. The frequency of abnormalities was significantly higher than in SARS-CoV-2 negative women (50%, $p < 0.05$). Demographic and obstetric data were similar in the SARS-CoV-2+ women across all sites - characterised by predominantly Black/Middle Eastern women, and women with elevated body mass index.
Discussion: Overall, the frequency of placental abnormalities is increased in SARS-CoV-2+ women, but the incidence of placental abnormalities is also higher in SARS-CoV-2- women that gave birth during the pandemic, which highlights the importance of appropriate control groups to ascertain the roles of pandemic stress and SARS-CoV-2 infection on the placenta and pregnancy outcomes.

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that appeared in late 2019 and has infected over 65.8 million people worldwide and is responsible for 1.5 million deaths [1]. Many studies have focused on the health impacts of the virus on vulnerable populations, including the elderly and those with chronic diseases since the virus has proven to be more deadly and to have a greater negative impact on these populations [2]. Overall, the SARS-CoV-2 pandemic has been associated with some reports of severe

* Corresponding author
E-mail address: sylvie.girard@umontreal.ca (S. Girard).

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Table 1
Demographic characteristics of the population studied.

Maternal characteristics	CANADA		UK		FRANCE
	SARS-CoV-2 + (N = 31)	SARS-CoV-2 - (N = 38)	SARS-CoV-2 + (N = 14)	SARS-CoV-2 - (N = 11)	SARS-CoV-2 + (N = 21)
Maternal age (years)	33 (26–45)	33 (18–42)	32 (21–45)	34 (25–40)	31 (21–43)
Ethnicity (%)					
Caucasian	7 (22.6)	28 (73.7)***	5 (35.7)	7 (63.6)	6 (40.0)
Black	9 (29.0)	3 (7.9)*	0 (0.0)*	3 (27.3)$^{0.0842,\#}$	2 (13.3)
Middle east	8 (25.8)	2 (5.3)*	7 (50.0)	1 (9.1)$^{\#}$	6 (40.0)
Others	7 (22.6)	5 (13.2)	2 (14.3)	0 (0.0)	1 (6.7)
BMI	32.6 (19.8–51.4)	24.9 (18.4–41.0)***	29.7 (21.0–42.1)	27.4 (17.6–42.8)	26.4 (20.0–33.0)*
Overweight (>25 < 30)	3 (13.0)	9 (25.7)	3 (21.4)	5 (45.5)	4 (30.7)
Obesity (>30)	15 (65.2)	4 (11.4)***	7 (50.0)	3 (27.3)	3 (23.1)*
Family history (HT & DM)	14 (45.2)	12 (31.6)	NA	NA	3 (14.2)*
Smoking (%)	1 (3.2)	4 (10.5)	1 (7.1)	2 (18.2)	0 (0.0)

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, BMI: body mass index, HT: hypertension, DM: diabetes mellitus; NA: Not available. Some information was unavailable: ethnicity: 6 missing – France; BMI: 11 missing Canada, 8 missing France). Data presented as mean (range) or n (%). * = $p < 0.05$, *** = $p < 0.001$ vs SARS-CoV-2 + CANADA; † = $p < 0.05$, vs SARS-CoV-2 – CANADA; # = $p < 0.05$ vs *SARS-CoV-2 + UK* by one-way ANOVA with Dunnett's multiple comparison post-test or Chi-square test as appropriate.

pregnancy complications, such as increased rates of spontaneous and iatrogenic preterm labor have been reported amongst COVID-19 patients, with rates of preterm birth of 17%, compared to the 8–10% reported in the general population [3–5].

The risk of vertical transmission of SARS-CoV-2 to the fetus is still unclear, but it has been reported [6], even though it is a rare event. There are a few studies that report placental infection, mostly through detection of viral particles and only within the maternal facing syncytiotrophoblast layer of the placenta [7], where vertical transmission has not been reported [8]. However, in adults, SARS-CoV-2 infection has been shown to induce most of its negative effect through inflammation - specifically through the "cytokine storm" detected in the patient's circulation [9–12], Importantly, systemic inflammation during pregnancy has been associated with an increased risk of pregnancy complications, such as preterm birth (PTB) [13,14]. Thus, even without direct viral infection, the placenta can still be severely impacted by maternal inflammation, but evidence is currently sparse about the risk associated with SARS-CoV-2 infection.

There have been some reports of the impact of SARS-CoV-2 infection on pregnancy, some including the placenta, and these were recently included in two systematic reviews [3,15]. So far, published work has reported increased thrombotic events, inflammation and vascular changes in the placenta from SARS-CoV-2 infected women [15]. The main limitation of these early studies is the lack of adequate control groups, specifically SARS-CoV-2 negative (−) women that were exposed to pandemic-specific stressors and that gave birth during the same period. Of high importance, stress during pregnancy has been shown to impact placental health and has been linked to inflammation, altered placental function and long-term changes in child development [16–19]. Thus, contemporaneous controls are required to ascertain changes resulting from SARS-CoV-2 infection versus those related to pandemic stress.

Our primary goal was to determine the effect of pandemic-related stressors, namely prenatal stress alone or combined with SARS CoV-2 infection, on placental histopathology. Importantly, we characterised a population of women infected with SARS-CoV-2 during pregnancy and performed in-depth histopathological analyses of the placentas across 5 sites in 3 countries (i.e. Canada, France and UK) using the same published placental analysis grid using the defined Amsterdam criteria [20]. We also determined pregnancy outcomes associated with SARS-CoV-2 and pandemic-related stress.

2. Methods

2.1. Study population

Placental samples (including fetal membranes and umbilical cord) from 115 women, who delivered between March and September 2020, were included in this study; 69 from Canada (61 from the Centre Hospitalier Universitaire (CHU) Sainte Justine-CHUSJ in Montreal, Quebec, and 8 from the Children Hospital of Eastern Ontario – CHEO, in Ottawa, Ontario); 21 from France (CHU Nancy and CHR Metz-Thionville) and 25 from the UK (St-Mary's Hospital, Manchester). This study was approved by each local research ethics board (REB - approval numbers - CHUSJ: MP-21-2019-1966; CHEO: 21/01X; CHU Nancy/CHR Metz-Thionville: TRANSCOVID study; St-Mary's Hospital: 15/NW/0829). We also included 20 historic controls from a Montreal-based cohort, who delivered between July 2016 and January 2019 (CHUSJ, REB No: MP-21-2019-1966). Details of each group (SARS-CoV-2 positive or negative) and from each site are given in Table 1.

Women were included based on a SARS-CoV-2 positive test (SARS-CoV-2 + group) or SARS-CoV-2 negative test (SARS-CoV-2 – group), by PCR on nasopharyngeal swab. The latter negative group was randomly selected within deliveries occurring during the same period as the SARS-CoV-2 positive cases at the CHUSJ, Canada and St Mary's Hospital, UK.

Demographic data such as maternal age, ethnicity, body mass index (BMI) and the medical history and obstetrical information regarding current pregnancy were collected through medical chart review.

2.2. Sample collection and histological analysis

Four villous tissue biopsies, including chorionic plate and decidua, across the placenta and a sample from the fetal membranes and umbilical cord were collected rapidly after delivery, fixed in 10% formalin (Sigma-Aldrich, MO, USA) for 5–7 days and paraffin-embedded and processed for histological analysis. Five micrometer thick sections were processed and stained with hematoxylin and eosin. Slides from the Manchester, UK; and Montreal, Canada; were all evaluated by the same pathologist blinded to group allocation to identify specific signatures and classified as: without abnormalities, structural defects, or inflammation. Furthermore, all sites used the standardized placental examination and lesion classification grid already published to perform an in-depth analysis of the placentas and based on the defined Amsterdam consensus [20,21]. Whole slide images were captured using a slide

Table 2
Obstetrical information of the current pregnancy.

Obstetrical characteristics	CANADA		UK		FRANCE
	SARS-CoV-2 + (N = 31)	SARS-CoV-2 - (N = 38)	SARS-CoV-2 + (N = 14)	SARS-CoV-2 - (N = 11)	SARS-CoV-2 + (N = 21)
Primiparity (%)	11 (35.5)	14 (36.8)	6 (42.9)	1 (9.1)$^{0.0786,0.0620}$	5 (23.8)
History of HT/DM (%)	7 (22.6)	0 (0.0)**	NA	NA	1 (4.8)
IVF (%)	1 (3.2)	2 (5.3)	NA	NA	0 (0.0)
GA at delivery (weeks)	38.7 (35.0–41.9)	39.4 (32.9–41.9)	38.8 (35.7–39.7)	38.5 (37.0–39.6)	38.8 (29.6–41.4)
Preterm birth (%)	5 (16.1)	3 (7.9)	1 (7.1)	0 (0.0)	2 (9.5)
Birthweight (grams)	3281 (890–4670)	3384 (1470–4350)	3270 (2700–4466)	3328 (2770–4250)	3028 (1450–3840)
HT current pregnancy (%)	8 (25.8)	3 (7.9)$^{0.0539}$	NA	NA	1 (4.8)$^{0.0670}$
DM current pregnancy (%)	7 (22.6)	4 (10.5)	2 (14.3)	NA	5 (23.8)
Induction of labor (%)	14 (45.2)	18 (47.4)	NA	NA	5 (23.8)
Delivery by CS (%)	10 (32.3)	8 (21.1)	9 (64.3)$^{0.0571}$	NA	2 (9.5)$^{0.0927,\#\#}$
Gender (% of male)	23 (74.2)	18 (47.4)*	7 (50.0)	5 (45.5)	15 (71.4)
SARS-CoV-2 Diagnosis (%)					
First trimester	0 (0.0)	–	NA	–	1 (4.8)
Second trimester	6 (19.4)	—		–	5 (23.8)
Third trimester	7 (22.6)			–	11 (52.4)
At delivery	17 (54.8)	–		–	4 (19.0)

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, HT: hypertension, DM: diabetes mellitus, IVF: *in vitro* fertilization, GA: gestational age, IBC: individualized birthweight centile, CS: cesarian-section, NA: Not available. Data presented as mean (range) or n (%). * = $p < 0.05$, ** = $p < 0.01$ vs SARS-CoV-2 + CANADA; † = $p < 0.05$, †† = $p < 0.01$, ††† = $p < 0.001$ vs SARS-CoV-2 – CANADA; ## = $p < 0.01$ vs *SARS-CoV-2 + UK* by one-way ANOVA with Dunnett's multiple comparison post-test or Chi-square test as appropriate.

Table 3
Placental macroscopic and histological analysis.

Placental analysis	CANADA		UK		FRANCE
	SARS-CoV-2 + (N = 31)	SARS-CoV-2 - (N = 38)	SARS-CoV-2 + (N = 14)	SARS-CoV-2 - (N = 11)	SARS-CoV-2 + (N = 21)
Placental weight (g)	529 (185–780)	478 (263–675)	566 (359–957)	612 (526–770)†	416 (205–681.)*,##
Placental percentile (%)					
<10	3 (9.7)	10 (26.3)	3 (21.4)	0 (0.0)$^{0.0902}$	9 (42.8)
[10–90[	18 (58.0)	23 (60.5)	6 (42.9)	7 (63.6)	11 (52.4)
>90	10 (32.3)	5 (13.2)$^{0.0791}$	5 (35.7)	4 (36.4)	1 (4.8)**,##
Macroscopic lesion (%)	4 (12.9)	10 (26.3)	NA	NA	7 (33.3)$^{0.0950}$
Microscopic abnormalities (%)					
None	6 (19.4)	18 (47.4)*	1 (7.1)	6 (54.5)#	3 (14.3)
Structural defect	22 (70.9)	17 (44.7)*	12 (85.7)	4 (36.4)#	17 (81.0)
Inflammation	7 (22.6)	7 (18.4)	2 (14.3)	1 (9.1)	2 (9.5)
Maternal vascular malperfusion (%)	7 (22.6)	6 (16.7)	2 (14.3)	1 (9.1)	11 (52.4)*,#
Fetal vascular malperfusion (%)	8 (25.8)	9 (23.7)	2 (14.3)	1 (9.1)	8 (38.1)
Maternal inflammatory response (%)	2 (6.5)	7 (18.4)	2 (14.3)	1 (9.1)	1 (4.8)
Fetal inflammatory response (%)	1 (3.2)	7 (18.4)$^{0.0655}$	NA	NA	NA
Villitis/perivillitis (%)	2 (6.5)	1 (2.6)	0 (0.0)	0 (0.0)	1 (4.8)
Deciduitis (%)	6 (19.4)	1 (2.6)*	0 (0.0)	0 (0.0)	1 (4.8)
Placental infarct (%)	1 (3.2)	3 (7.9)	0 (0.0)	0 (0.0)	2 (9.5)
Thrombosis (%)	5 (16.1)	3 (7.9)	1 (7.1)	0 (0.0)	6 (28.6)
Accelerated villous maturation (%)	5 (16.1)	0 (0.0)*	1 (7.1)	1 (9.1)	1 (4.8)
Excess syncytial knots (%)	3 (9.7)	3 (8.3)	0 (0.0)	0 (0.0)	11 (52.4)**,###
Excess fibrin (%)	17 (54.8)	21 (55.3)	7 (50.0)	1 (9.1)†,#	8 (38.1)
Avascular villi (%)	2 (6.5)	5 (13.2)	0 (0.0)	1 (9.1)	2 (9.5)
Calcifications (%)	10 (32.3)	8 (21.1)	12 (85.7)**	1 (9.1)###	0 (0.0)**,###
Agglutination (%)	2 (6.5)	0 (0.0)	1 (7.1)	0 (0.0)	6 (28.6)*
Congestive (%)	6 (19.4)	9 (23.7)	2 (14.3)	2 (18.2)	NA
Chorangiosis (%)	2 (6.5)	2 (5.3)	0 (0.0)	0 (0.0)	11 (52.4)***,##
Villous hypoplasia (%)	1 (3.2)	1 (2.6)	0 (0.0)	0 (0.0)	3 (14.3)

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, NA: Not available. Data presented as mean (range) or n (%). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ vs SARS-CoV-2 + CANADA; † = $p < 0.05$ vs SARS-CoV-2 – CANADA; # = $p < 0.05$, ## = $p < 0.01$, ### = $p < 0.001$ vs *SARS-CoV-2 + UK* by one-way ANOVA with Dunnett's or Chi-square test as appropriate.

scanner and pictures were taken for visualization (Axioscan, Zeiss, ON, Canada; Pannoramic ScanII, 3D Histech, Hungary), or images were taken using a microscope (Leica microscope mounted with a Leica DMC 2900 camera, Leica, France).

2.3. Statistical analysis

Data are presented as mean ± standard error of the mean or percentage. Data were analysed using one-way ANOVA with Dunnett's multiple comparisons post-test or Fisher's exact test, as appropriate. Multivariate logistic regression analysis was performed to assess the relationship between the presence of placental abnormalities and SARS-CoV-2 subjects including the country of origin as a variable. As no difference was observed in any of the parameters studied between women from both sites in Canada (CHUSJ and CHEO), they were combined for analysis. Statistical analysis was performed with GraphPad Prism 8.1.2 (GraphPad Software, CA) or SAS 9.4 and a p-value of <0.05 was considered statistically significant.

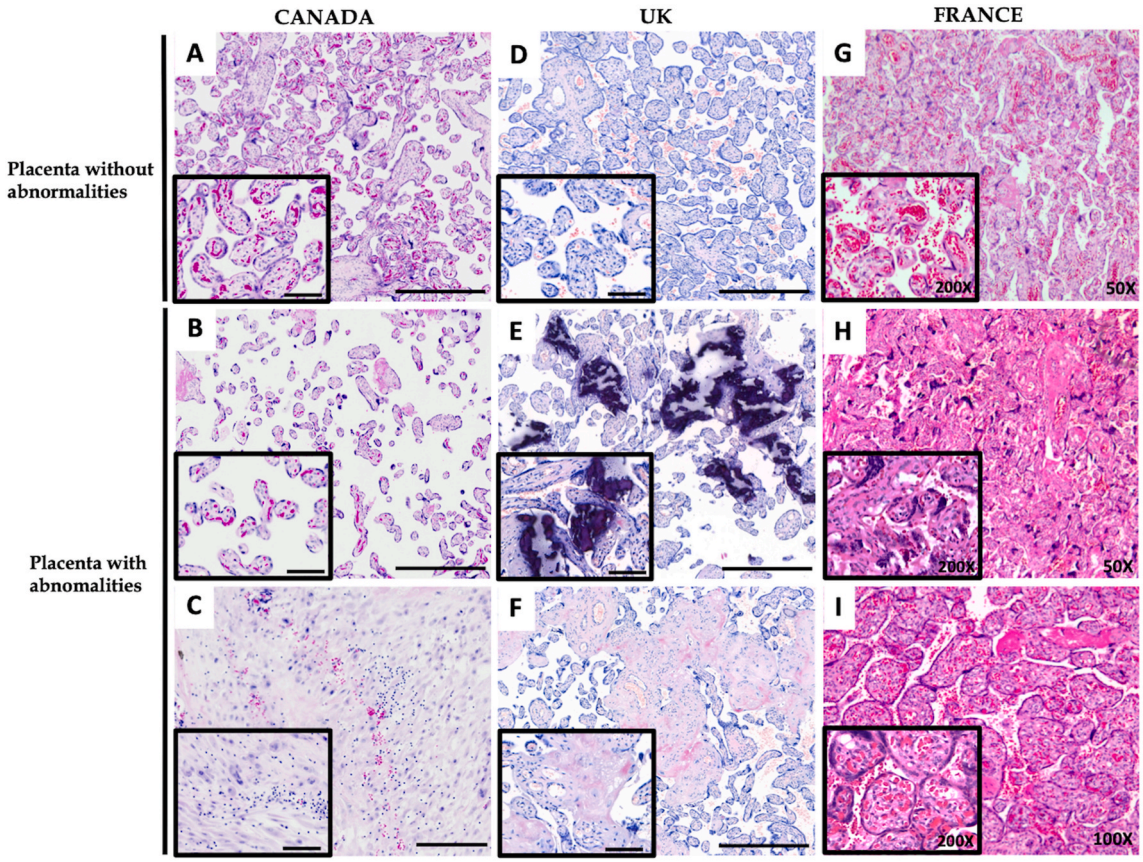

Fig. 1. Example of placental abnormalities observed in placentas from SARS-CoV-2+ pregnancies. Representative images of normal histopathology (A, D, G). The predominant abnormalities observed in the Canada cohort were accelerated villous maturation (B) and deciduitis (C); in the UK cohort, calcifications (E) and excess fibrin (F); whilst in the France cohort excess syncytial knots (H) and chorangiosis (I) were observed. Hematoxylin-eosine staining. Scale bar in A, B, D, E, F are 500 μm and higher magnification 100 μm. Scale bar in C: 200 μm and higher magnification 100 μm.

3. Results

We first compared the maternal demographic and pregnancy characteristics of SARS-CoV-2+ women from the 3 sites: Canada (N = 31), UK (N = 14) and France (N = 21) with SARS-CoV-2- women from Canada (N = 38) and UK (N = 11) (shown in Table 1). In all sites, women infected with SARS-CoV-2 were of diverse multiethnic origins, compared to a majority of Caucasian women in the SARS-CoV-2– groups (Table 1). Maternal body mass index (BMI) was elevated in SARS-CoV-2+ women only in the Canadian cohort. Furthermore, women in the Canadian cohort had a higher proportion of family history of hypertension and diabetes as opposed to the France group, whilst these data were not obtained for the UK cohort. No difference was observed in the other demographic characteristics evaluated between the SARS-CoV-2+ women in the UK, Canada or France. Obstetric characteristics are shown in Table 2. Overall, there were no major differences observed between the 3 cohorts. The only observed differences were associated with SARS-CoV-2 infection, which was associated with hypertension in the current pregnancy (25.8% vs 7.9% in SARS-CoV-2-, p = 0.05) and personal history of hypertension/diabetes (22.6% vs 0% in SARS-CoV-2-, p = 0.01), which were only observed in the Canadian cohort. There was also a slight, but not statistically significant, increased rate of preterm birth (16.1% in SARS-CoV-2+ vs 7.9% in SARS-CoV-2-).

Several placental changes were observed in women with SARS-CoV-2 infection, shown in Table 3. In all cohorts, over 75% of placentas from SARS-CoV-2+ pregnancies presented microscopic abnormalities, either structural or inflammatory, which was higher than what was observed in placentas from SARS-CoV-2- pregnancies. Multivariable logistic regression was used to assess the association between SARS-CoV-2 infection, study site (country of origin) and the presence of histopathological placental abnormalities. Only samples from Canada and the UK were included in this analysis, since these sites had samples from both SARS-CoV-2 positive and negative patients. The probability that SARS-CoV-2 infection affected the placenta leading to structural or inflammatory abnormalities were both significant (p = 0.0019 and p = 0.038 respectively, both countries combined), whilst no difference was observed related to the country (p = 0.72 and p = 0.73).

The observed placental abnormalities were predominantly structural across all cohorts (71% in Canada; 86% in the UK; 81% in France). Within these structural defects in placentas from SARS-CoV-2+ pregnancies, accelerated villous maturation was observed in the Canadian cohort, whilst excess fibrin and calcifications were observed predominantly in the UK cohort (Table 3, Fig. 1 and Fig. 2A and B). Within the France cohort, excess syncytial knots and agglutinations were observed, as compared to both SARS-CoV-2- cohorts (Canada and UK) (Table 3, Figs. 1 and 2C). Both maternal and fetal vascular malperfusion were observed, predominantly in the Canadian cohort, however, these were observed in both SARS-CoV-2 ± groups of patients. Inflammatory changes within the placenta were observed, especially in the Canadian cohort and were characterised by deciduitis (Table 3 and Fig. 1). We also analysed the presence of multiple types of lesions within each placenta and observed that half (6 out of 12) of the placentas with inflammatory lesions also had structural abnormalities (Fig. 2). Furthermore, excess perivillous fibrin was observed across all sites studied (Fig. 2).

Further in-depth analysis of the Canadian cohort was performed to compare placentas from symptomatic and asymptomatic SARS-CoV-2+ women. Interestingly, all demographic and obstetrical characteristics were similar in both groups, with the exception of the rate of PTB, which

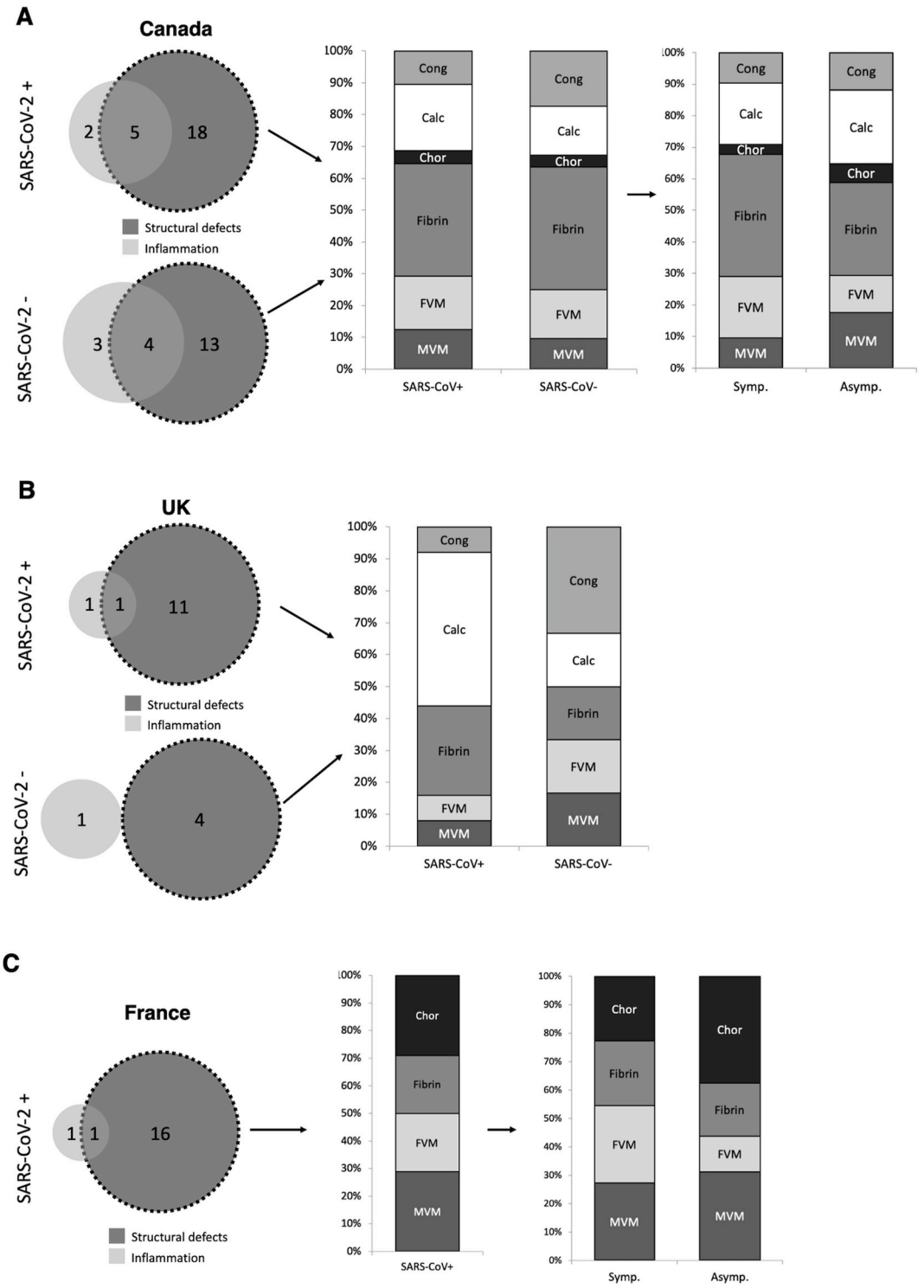

Fig. 2. Placental lesions distribution across all sites. Graphical representation of the lesion distribution in Canada (A), UK (B) and France (C) showing the overlapping presentation of structural and inflammatory defects and the percentage of each subtype of structural defect. Cong: congestive; Calc: calcification; Chor: chorangiosis; FVM: fetal vascular malperfusion; MVM: maternal vascular malperfusion.

was more prevalent in the symptomatic subgroup (22.2 vs 7.6% in asymptomatic) (Table 4). Regarding the placental analysis, the only difference observed was the lower placental weight in the symptomatic group (480 vs 597g; $p = 0.053$) whilst the seemingly elevated rate of deciduitis and excess fibrin were not significant (Table 5).

We additionally analysed 20 historic controls alongside the SARS-CoV-2 ± samples and these further confirmed the elevated BMI and higher proportion of hypertension in women recruited during the pandemic (Table 4). For the placental analysis, the comparison with historical controls further showcased the importance of the observed placental microscopic abnormalities, which were absent in 75% of the historic controls (Table 5). Both maternal and fetal vascular malperfusion, as well as congestion and excess fibrin, were only observed in the SARS-CoV-2+/− populations, and significantly elevated incidence versus historic controls. On the other hand, deciduitis and accelerated villous maturation were observed specifically in SARS-CoV-2+

Table 4
Maternal and obstetrical characteristics in symptomatic vs asymptomatic SARS-CoV-2+ patient and comparison with Historic control in the Canadian cohort only.

Maternal and obstetrical characteristics	SARS-CoV-2 +			SARS-CoV-2 -(N = 38)	Historic control (N = 20)
	Symptomatic (N = 18)	Asymptomatic (N = 13)	All (N = 31)		
Maternal age (years)	33 (26–45)	34 (26–41)	33 (26–45)	33 (18–42)	35 (23–42)
Ethnicity (%)					
Caucasian	6 (33.4)	1 (7.7)	7 (22.6)***,0.0676	28 (73.7)0.0876	10 (50.0)
Black	4 (22.2)	5 (38.5)	9 (29.0)*	3 (7.9)0.0516	6 (30.0)
Middle east	4 (22.2)	4 (30.8)	8 (25.8)*	2 (5.3)	2 (10.0)
Others	4 (22.2)	3 (23.0)	7 (22.6)	5 (13.2)	2 (10.0)
BMI	33.1 (19.8–51.4)	31.8 (20.2–46.3)	32.6 (19.8–31.6)***,#	24.9 (18.4–41.0)	25.9 (18.7–39.0)
Overweight (>25, <30)	2 (14.3)	1 (11.1)	3 (13.0)	9 (25.7)	5 (25.0)
Obesity (>30)	9 (64.3)	6 (66.7)	15 (65.2)***,#	4 (11.4)	5 (25.0)
Family history (HT & DM)	8 (44.4)	6 (46.2)	14 (45.2)	12 (31.6)	9 (45.0)
Smoking (%)	1 (5.6)	0 (0.0)	1 (3.2)	4 (10.5)	0 (0.0)
Primiparity (%)	10 (55.6)	1 (7.7)††	11 (35.5)	14 (36.8)	4 (20.0)
History of HT/DM (%)	3 (16.7)	4 (30.8)	7 (22.6) **,#	0 (0.0)	0 (0.0)
IVF (%)	1 (5.6)	0 (0.0)	1 (3.2)	2 (5.3)	1 (5.0)
GA at delivery (weeks)	38.6 (35.0–41.9)	38.9 (36.4–40.9)	38.7 (35.0–41.9)	39.4 (32.9–41.9)	39.7 (38.0–41.1)
Preterm birth (%)	4 (22.2)	1 (7.6)	5 (16.1)	3 (7.9)	0 (0.0)
Birthweight (grams)	3239 (890–4560)	3337 (2280–4670)	3281 (890–4670)	3384 (1470–4350)	3525 (2960–4080)
HT current pregnancy (%)	4 (22.2)	4 (30.8)	8 (25.8)*,#	3 (7.9)	0 (0.0)
DM current pregnancy (%)	1 (5.6)	6 (46.2)†	7 (22.6)	4 (10.5)	4 (20.0)
Induction of labor (%)	9 (50.0)	5 (38.5)	14 (45.2)	18 (47.4)	7 (35.0)
Delivery by CS (%)	5 (27.8)	5 (38.5)	10 (32.3)0.0819	8 (21.1)##	12 (60.0)
Gender (% of male)	14 (77.8)	9 (69.2)	23 (74.2)*	18 (47.4)	10 (50.0)

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, BMI: body mass index, HT: hypertension, DM: diabetes mellitus, IVF: *in vitro* fertilization, GA: gestational age, IBC: individualized birthweight centile, CS: cesarian-section. Some information was unavailable in the BMI category (4 in the symptomatic; 4 in the asymptomatic; 3 in the negative groups). Data presented as mean (range) or n (%). † = $p < 0.05$, †† = $p < 0.01$ vs *SARS-CoV-2 + Symptomatic*; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ vs SARS-CoV-2 -, # = $p < 0.05$ vs Historic control by one-way ANOVA with Dunnett's multiple comparison post-test or Chi-square test as appropriate.

Table 5
Placental analysis in symptomatic vs asymptomatic SARS-CoV-2+ patient and comparison with Historic control in the Canadian cohort only.

Placental analysis	SARS-CoV-2 +			SARS-CoV-2 -	Historic control
	Symptomatic (N = 18)	Asymptomatic (N = 13)	All (N = 31)	(N = 38)	(N = 20)
Placental weight (g)	480 (185–664)	597 (357–780)0.0530	529 (185–780)	478 (263–675)	479 (320–754)
Placental percentile (%)					
<10	2 (11.1)	1 (7.7)	3 (9.2)#	10 (26.3)	7 (35.0)
[10–90[	12 (66.7)	6 (46.2)	18 (58.0)	23 (60.5)	10 (50.0)
>90	4 (22.2)	6 (46.2)	10 (32.3)0.0791	5 (13.2)	3 (15.0)
Macroscopic lesion (%)	1 (5.6)	3 (23.1)	4 (12.9)	10 (26.3)0.0772	1 (5.0)
Microscopic abnormalities (%)					
None	3 (16.7)	3 (23.1)	6 (19.4)*,###	18 (47.4)0.0546	15 (75.0)
Structural defect	14 (77.8)	8 (61.5)	22 (70.9)*,###	17 (44.7)#	3 (15.0)
Inflammation	5 (27.8)	2 (15.4)	7 (22.6)	7 (18.4)	3 (15.0)
Maternal vascular malperfusion (%)	4 (22.2)	3 (23.1)	7 (22.6)#	6 (16.7)0.0837	0 (0.0)
Fetal vascular malperfusion (%)	6 (33.3)	2 (15.4)	8 (25.8)	9 (23.7)	2 (10.0)
Maternal inflammatory response (%)	2 (11.1)	0 (0.0)	2 (6.5)	7 (18.4)	1 (5.0)
Fetal inflammatory response (%)	1 (5.6)	0 (0.0)	1 (3.2)*	7 (18.4)0.0834	0 (0.0)
Villitis/perivillitis (%)	2 (11.1)	0 (0.0)	2 (6.5)	1 (2.6)	0 (0.0)
Deciduitis (%)	4 (22.2)	2 (15.4)	6 (19.4)*	1 (2.6)	2 (10.0)
Placental infarct (%)	1 (5.6)	0 (0.0)	1 (3.2)	3 (7.9)	0 (0.0)
Thrombosis (%)	3 (16.7)	2 (15.4)	5 (16.1)	3 (7.9)	2 (10.0)
Accelerated villous maturation (%)	2 (11.1)	3 (23.1)	5 (16.1)*	0 (0.0)	0 (0.0)
Excess syncytial knots (%)	1 (5.6)	2 (15.4)	3 (9.7)	3 (8.3)	0 (0.0)
Excess fibrin (%)	12 (66.7)	5 (38.5)	17 (54.8)#	21 (55.3)0.0505	5 (25.0)
Avascular villi (%)	2 (11.1)	0 (0.0)	2 (6.5)	5 (13.2)	0 (0.0)
Calcifications (%)	6 (33.3)	4 (30.8)	10 (32.3)	8 (21.1)	3 (15.0)
Agglutination (%)	2 (11.1)	0 (0.0)	2 (6.5)	0 (0.0)	0 (0.0)
Congestive (%)	4 (22.2)	2 (15.4)	6 (19.4)0.0697	9 (23.7)#	0 (0.0)
Chorangiosis (%)	1 (5.6)	1 (7.7)	2 (6.5)	2 (5.3)	0 (0.0)
Villous hypoplasia (%)	1 (5.6)	0 (0.0)	1 (3.2)	1 (2.6)	0 (0.0)

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; Data presented as mean (range) or n (%). † = $p < 0.05$, †† = $p < 0.01$, ††† = $p < 0.001$ vs *SARS-CoV-2 + Symptomatic*; * = $p < 0.05$ vs SARS-CoV-2 -, # = $p < 0.05$, ### = $p < 0.001$ vs Historic control by one-way ANOVA with Dunnett's multiple comparison post-test or Chi-square test as appropriate.

pregnancies, whilst maternal and fetal inflammatory responses were observed specifically in the SARS-CoV-2- population.

4. Discussion

We studied placentas from SARS-CoV-2+ pregnancies, alongside placentas from SARS-CoV-2- pregnancies, which occurred during the same period (meaning that women were exposed to pandemic-related stress with or without infection) across 5 different sites in 3 countries (i.e. Canada, France and UK). Across all cohorts, we observed that over 75% of placentas had histopathological abnormalities (Canada: 80.6%, UK: 92.9%, France: 87.5%), which were significantly higher than the SARS-CoV-2- samples (Canada: 52.6%, UK: 45.5%). Using multivariate logistic regression analysis, we showed that the country of origin was not associated to placental abnormalities whilst the SARS-CoV-2 status (i.e. positive vs negative) was significantly associated with both structural and inflammatory abnormalities. These were much higher than in historic controls (placentas from pregnancies prior to the pandemic, therefore not exposed to pandemic-related stress) from which 25% presented histological abnormalities. Although our historic control group was small (n = 25), our work is in full accordance with a previous report, where approximately 25% of placentas from a large cohort of over 1000 placentas, presented lesions [22]. These observations suggest a strong effect of pandemic-related stress and an added impact of SARS-CoV-2 infection during pregnancy.

The SARS-CoV-2+ women recruited in Canada had elevated BMI and increased rates of PTB, especially in symptomatic women, and increased hypertension during their pregnancy. This was not observed in the UK or France cohorts, even if the observed placental effects were similar. These characteristics of infected women observed in our Canadian cohort were similar to published work from the USA [23] and could suggest differences in patient susceptibilities, potentially due to related family histories between these women (elevated history of hypertension and diabetes). These findings are consistent with the reported social and racial inequities of the pandemic observed in populations other than pregnant women [24,25].

Of interest, the high proportion of placentas presenting abnormalities in SARS-CoV-2+ pregnancies were observed in all 3 sites. Of these abnormalities, maternal and fetal vascular malperfusion were most frequently observed, as previously reported [15], although to a lesser extent than formerly published work [26,27] and was observed predominantly in the Canadian and France cohorts. Interestingly, these abnormalities were also observed in the SARS-CoV-2- population, which suggests an important component of stress. Another study reported no significant differences in maternal vascular malperfusion between SARS-COV-2 ± women (48% vs 20%) - potentially due to the higher than normal presentation in the SARS-CoV-2- cohort, which was exposed to pandemic related stressors [28]. Maternal and fetal inflammatory responses were observed in a small proportion of placentas from SARS-CoV-2+ pregnancies but were also seen in the Canada SARS-CoV-2- population, which strongly supports the effect on the placenta of pandemic-related stressors, even without infection. This is in line with previous studies of the negative impact of stress during pregnancy on the placenta. The other sign of inflammation observed was deciduitis, which was seen in the Canadian SARS-CoV-2+ population but not in the UK or France cohorts. Evidence of placental inflammation related to SARS-CoV-2 infection has been sparse, with some extreme cases of placentitis [29] but most reporting no to moderate changes [15,26]. A further in-depth investigation is still required, whilst taking into account the potential contribution of prenatal stress.

Within the observed structural placental abnormalities, excess fibrin was observed across all sites and is also prevalent in the reported studies of placentas from SARS-CoV-2+ pregnancies [26,30,31]. Excess fibrin was also observed in the Canadian SARS-CoV-2- cohort, but much less frequently in the negative cohort from the UK. The actual relationship between SARS-CoV-2 infection and fibrin deposition in the placenta remains to be investigated. Calcifications were prevalent in the SARS-CoV-2+ UK population, whilst excess syncytial knots and chorangiosis were observed solely in the France cohort. These remain to be further investigated in larger cohorts, but it is important to note that there were no major differences within the populations of each cohort that could explain these differences.

4.1. Strengths and limitations

The strengths of this study are the multicentre and international component, with all analysis were performed using standardised diagnostic criteria, which together allowed the understanding of the impact of pandemic related stress with and without the added SARS-CoV-2 infection on the placenta. On the other hand, the study has some limitations, such as the relatively small number of participants included, and likely many asymptomatic pregnant women not screened at the beginning of the pandemic - potentially leading to an overestimation of the frequency of lesions in the SARS-CoV-2+ population. Although we used multivariate logistic regression analysis to assess the contribution of different sites and SARS-CoV-2 status to the placental abnormalities, the small number of patients did not allow for further analysis of the contribution of other factors such as maternal comorbidities. It is also possible that part of the findings in the SARS-CoV-2+ population was related to other pathologies (i.e. preeclampsia, placental insufficiency).

Our study does not have the statistical power to differentiate between trimester of exposure to SARS-CoV-2 infection, but this would be a very important point to address in future work. Some of the observed lesions, such as fibrin deposition and calcifications, are often related to chronic rather than acute placental exposure, and it will be of high importance to address this, especially as the SARS-CoV-2 pandemic has now been among us for over a year. Another very important point is the possible cumulative impact of psychological and infectious stress on the placenta. Our study, and others, definitely support that both stressors play definite roles, but the extent of each implication remains to be defined. Lastly, even if it has been reported for many other medical fields [32,33], no significant change in prenatal care has been observed in the institutions included in this study (no delay/avoidance of medical care).

5. Conclusions

Globally our results indicate that placental abnormalities have a high prevalence in SARS-CoV-2+ patients. Of high importance, the incidence of placental abnormalities is significantly higher in SARS-CoV-2- women that delivered during the pandemic versus historic controls. This highlights the importance of appropriate control groups, in this particular case, to ascertain the role of both pandemic-related stress and SARS-CoV-2 infection itself on the placenta and pregnancy outcomes. Future studies will be needed to understand both the short and long-term impacts of SARS-CoV-2 infection and pandemic stress on the neonate, as well as on maternal health, and also to understand if infection earlier in pregnancy has a different impact on pregnancy and neonatal outcomes.

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Declaration of competing interest

None.

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