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Third Trimester Placentas of SARS-CoV-2-Positive Women: Histomorphology, including Viral Immunohistochemistry and in Situ Hybridization.

Short running title: Placentas in SARS-CoV-2-Positive Women

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Abstract

Aims: The wide-variety of affected organ-systems associated with SARS-CoV-2 infection highlights the need for tissue-specific evaluation. We compared placentas from SARS-CoV-2-positive and negative women in our hospital in New York City, which became the epicenter of the COVID-19 pandemic in March 2020. While some limited studies have been published on placentas from SARS-CoV-2-positive women to date, this study, in addition to describing histomorphology, utilizes in-situ hybridization (ISH) for the S-gene encoding the spike-protein and immunohistochemistry (IHC) with the monoclonal-SARS-CoV-2 spike-antibody 1A9 for placental evaluation.

Methods and Results: In this study, 51 singleton, third-trimester placentas from SARS-CoV-2-positive women and 25 singleton, third-trimester placentas from SARS-CoV-2-negative women were examined histomorphologically using the Amsterdam Criteria as well as with ISH and/or IHC. Corresponding clinical findings and neonatal outcomes also were recorded. While no specific histomorphologic changes related to SARS-CoV-2 were noted in the placentas, evidence of maternal/fetal vascular malperfusion was identified, with placentas from SARS-CoV-2-positive women significantly more likely to show villous agglutination ($p=0.003$) and subchorionic thrombi ($p=0.026$) than placentas from SARS-CoV-2-negative women. No evidence of direct viral involvement was identified using ISH and IHC.

Conclusions: In this study, third trimester placentas from SARS-CoV-2-positive women were more likely to show evidence of maternal/fetal vascular malperfusion; however, no evidence of direct viral involvement or vertical transmission was noted by ISH and IHC.

Key Words: Pregnancy; Pregnancy Trimester, Third; COVID-19; SARS-CoV-2; Placental Pathology; Immunohistochemistry; In Situ Hybridization

Introduction:

An outbreak of pneumonia caused by the 2019 novel coronavirus (SARS-CoV-2), first reported in Wuhan, China[1] in December 2019, rapidly spread throughout the world and was declared a pandemic by The World Health Organization (WHO) on March 11, 2020. This led many hospitals, especially within SARS-CoV-2 epicenters, to initiate universal SARS-CoV-2 testing of women presenting to labor and delivery (L&D). At our academic hospital, Columbia University Irving Medical Center (CUIMC) in New York City, preliminary data showed that SARS-CoV-2 testing was positive in 15.4% of women presenting to L&D, 87.9% of whom were asymptomatic.[2]

Pneumonias arising from any infectious etiology are an important cause of morbidity and mortality among pregnant women. Adverse outcomes include premature rupture of membranes, preterm labor, intrauterine fetal demise, intrauterine growth restriction, and neonatal death.[3] Though most SARS-CoV-2 cases are mild to moderate, severe disease, termed COVID-19, is devastating many communities. Many questions regarding its pathophysiology and impact on specific populations, including pregnant women, remain. Studies to date regarding SARS-CoV-2 and placental pathology have been limited by the number of SARS-CoV-2 positive cases,[4,5] and only one with a sample size of 5 cases has utilized in-situ hybridization (ISH) and immunohistochemistry (IHC) analyses.[6] In our study, we compared placental histopathology from 51 SARS-CoV-2-positive and 25 SARS-CoV-2-negative women in their third-trimesters presenting to L&D, and tested placentas from SARS-CoV-2-positive mothers using ISH and/or IHC.

Materials and Methods:

For this study, we examined placentas from 51 SARS-CoV-2-positive women, as documented in their electronic medical records, from 3/23/20 to 4/29/20, the peak of SARS-CoV-2 infection in New York City. Twenty-five selected consecutively received singleton

third trimester placentas from SARS-CoV-2-negative women from the same time period were reviewed for comparison. All mothers were tested in L&D via nasopharyngeal swabs, using a RT-PCR test [2]. All neonates born to SARS-CoV-2-positive mothers were tested for SARS-CoV-2 immediately after birth using the same methodology.

All placentas were grossly examined and H&E-stained sections of umbilical cords, membranes, and discs, including fetal and maternal plates, were reviewed. The placentas from SARS-CoV-2-positive mothers were tested using in-situ hybridization (ISH) for the S-gene encoding the spike-protein (RNAscope-ProbeV-nCoV2019-S; Advanced-Cell-Diagnostics, Hayward, CA) according to the manufacturer's protocol and/or immunohistochemistry (IHC) with the monoclonal-SARS-CoV-2 spike-antibody 1A9, 1:1000 dilution (GeneTex, Irvine, CA). Both ISH and IHC utilized on-slide positive controls of lung tissue from SARS-CoV-2-positive autopsy specimens. The 51 placentas from SARS-CoV-2-positive mothers were tested with ISH and/or IHC: 32 with both ISH and IHC, 5 with ISH only, and 14 with IHC only. Slides were re-reviewed in consensus using the Amsterdam criteria[7]. Fischer's exact test was performed to examine differences between morphologic features in placentas from SARS-CoV-2-positive and SARS-CoV-2-negative women. The CUIMC Institutional Review Board approved this study.

Results:

Clinical Data

Of the 76 placentas in the study, maternal ages ranged from 19 to 47 years (mean: 29.2±6.1) for SARS-CoV-2-positive and 21 to 42 (mean: 32.3 ±5.7) for SARS-COV-2-negative mothers. Of the 51 SARS-CoV-2-positive mothers, 51% (26) were asymptomatic. Cough (61.5%), fever (53.8%), myalgia (26.9%), sore throat (11.5%) and fatigue (11.5%) were the most common symptoms. Although the majority of patients were either asymptomatic or exhibited mild symptoms, four (15.4%) had severe disease necessitating supplemental oxygen, treatment with experimental therapies (hydroxychloroquine, azithromycin, tocilizumab, Remdesivir) and, in one patient, intubation. Comorbidities, including obesity, hypertension, preeclampsia, diabetes, hypothyroidism and asthma, were

similar between SARS-CoV-2-positive and SARS-COV-2-negative mothers. Delivery methods and preterm delivery (<37 weeks of gestational age) also were similar between these two groups (Table 1). No adverse perinatal outcomes were identified: all neonates from SARS-CoV-2-positive mothers had high (>7) 5-minute Apgar scores. No deaths were reported.

Placental Pathology

The majority of placental weights and fetal-placental weight ratios for SARS-CoV-2-positive and SARS-COV-2-negative women were within 10^{th}-90^{th} percentile reference ranges. Placentas from SARS-CoV-2-positive women showed non-specific evidence of maternal/fetal vascular malperfusion, including subchorionic thrombi (Fig.1A), intervillous thrombi (Fig.1B), infarction (Fig.1C), chorangiosis, segmental avascular-villi (Fig.1D), fetal thrombotic vasculopathy (Fig.1E), and villous agglutination (Fig.1F). Of these, villous agglutination and subchorionic thrombi were significantly more likely to occur in placentas from SARS-CoV-2-positive women than SARS-CoV-2-negative women (p=0.026 and p=0.003, respectively). We found no statistically significant differences between placentas from symptomatic and asymptomatic SARS-CoV-2 positive women in terms of intervillous thrombi, infarction, chorangiosis, accelerated villous maturation, etc. (Table 2). No lesions associated with direct viral involvement (viral cytopathic changes) were noted, and both ISH (Fig, 1G) and IHC (Fig. 1H) testing were negative in all tested placentas from SARS-CoV-2-positive mothers. All neonates born to SARS-CoV-2-positive mothers tested negative for SARS-CoV-2.

Discussion:

SARS-CoV-2 infects target cells by binding to angiotensin-converting enzyme II (ACE2) via its surface spike protein in a similar fashion to the SARS-CoV virus responsible for the epidemic of Severe Acute Respiratory Syndrome (SARS) in 2002-2003[8]. ACE2 has been reported to be present in decidual cells, syncytiotrophoblasts, cytotrophoblasts, and endothelium and vascular smooth muscle of primary and secondary villi.[9,10] While the presence of placental ACE2 suggests the possibility of SARS-CoV-2 infection of these cells,

we did not see direct viral presence of SARS-CoV-2 in placentas by morphology, IHC, and ISH targeting the spike protein, similar to other studies.[6]

Thus far, studies have reported similar clinical symptoms and outcomes between SARS-CoV-2-positive pregnant and SARS-CoV-2-positive non-pregnant women.[11] One report describes mortality of a SARS-CoV-2-positive pregnant woman.[12] However, to date, there have been no reported cases of definitive vertical transmission.[13-15] Studies from the previous SARS outbreak indicated that SARS was associated with higher incidences of spontaneous miscarriage, preterm delivery, and intrauterine growth restriction, but without vertical-transmission.[16] Placental pathologic features described in association with SARS, e.g., increases in intervillous or subchorionic fibrin and avascular fibrotic villi,[17] are similar to our histomorphologic findings. Compared with SARS-CoV-2-negative placentas, in the SARS-CoV-2-positive placentas there was an increased incidence of villous agglutination, subchorionic thrombi, accelerated villous maturity, chorangiosis, fetal thrombotic vasculopathy, and avascular villi, suggestive of fetal stress and warranting further investigation (Table 1). In view of the fact that retroplacental and intraplacental hemorrhages are considered evidence of maternal vascular malperfusion (MVM) according to the Amsterdam Criteria and other studies,[18-21] we have classified subchorionic thrombi as evidence of MVM in our study.

Placental weight (PW) is closely related to fetal growth and is affected by various pregnancy-related conditions.[22] The fetal-placental weight ratio (FPR) is commonly used to assess the possibility of underlying pathologic conditions and poor perinatal outcomes.[23] In our study, the majority of PWs and FPR from SARS-CoV-2-positive and SARS-CoV-2-negative mothers were within the 10th and 90th percentile reference range.[24]

In our limited study of 51 placentas from SARS-CoV-2-positive mothers in their third trimesters, we found no definite evidence of SARS-CoV-2 in the placentas by ISH and IHC, and we noted nonspecific histomorphologic changes suggestive of maternal/fetal vascular malperfusion. All neonates tested negative for SARS-CoV-2, and all mothers recovered clinically. Further studies, including more sensitive techniques for viral infection (e.g., RT-PCR), are warranted.

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Author contributions:

Marie C. Smithgall, Diane Hamele-Bena, and Xiaowei Chen: Conception, design, primary acquisition, data analysis, and writing of the manuscript.

Xiaolin Liu-Jarin, Adela Cimic, Larisa Debelenko: Conception, design, and data acquisition

Mirella Mourad: Clinical data acquisition.

References:

1. Zhu N, Zhang D, Wang W, *et al*. A novel coronavirus from patients with pneumonia in China. New England Journal of Medicine 2020; 382: 727-733.

2. Sutton D, Fuchs K, D'Alton M, Goffman D. Universal screening for SARS-CoV-2 in women admitted for delivery. New England Journal of Medicine 2020; DOI:10.1056/nejmc.2009316

3. Schwartz DA and Graham A. Potential maternal and infant outcome from coronavirus 2019-nCoV (SARS-CoV-2) infecting pregnant women: Lessons from SARS, MERS, and other human coronavirus infections. Viruses 2020; DOI: 10.3390/v12020194

4. Baergen RN, Heller DS. Placental Pathology in Covid-19 Positive Mothers: Preliminary Findings. Pediatric and Developmental Pathology 2020; 23:177-180.

5. Shanes ED, Mithal LB, Otero S, Azad HA, Miller ES, Goldstein JA. Placental Pathology in COVID-19. American Journal of Clinical Pathology 2020; 154:23-32.

6. Mulvey JJ, Magro CM, Ma LX, Nuovo GJ, Baergen RN. Analysis of complement deposition and viral RNA in placentas of COVID-19 patients. Annals of Diagnostic Pathology 2020; DOI: 10.1016/j.anndiagpath151530

7. Khong TY, Mooney EE, Ariel I, Balmus NC, *et al.* Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Archives of Pathology and Laboratory Medicine 2016; 140: 698-713.

8. Lu R, Zhao X, Li J, *et al*., Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origin and receptor binding. Lancet 2020; 395: 565-574.

9. Hamming, W. Timens, Bulthuis MJ, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. Journal of Pathology 2004; 203: 631-637.

10. Valdes G, Neves LA, Anton L, *et al*. Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta 2006; 27: 200-207.

11. Chen H, Gui J, Wang C, *et al*. Clinical characteristics and intrauterine vertical transmission potential of COVD-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 2020; 395: 809-815.

12. Karami P, Naghavi M, Feyzi A, *et al*. Mortality of a pregnant patient diagnosed with COVID-19: a case report with clinical, radiological, and histopathological findings. Travel Medicine and Infectious Disease 2020; DOI:10.1016/j.tmaid101665

13. Chen Y, Peng H, Wang L, *et al*., Infants born to mothers with a new coronavirus (COVID-19). Frontiers in Pediatrics 2020; 8: 104.

14. Chen H, Gui J. Wang C, *et al*. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 2020; 395: 809-815.

15. Zhu H, Wang L, Fang C, *et al*. Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. Translational Pediatrics 2020; 9: 51-60.

16. Wong SF, Chow KM, Leung TN, *et al*. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. American Journal of Obstetrics and Gynecology 2004; 191: 292-297.

17. Ng WF, Wong SF, Lam A, *et al*. The placentas of patients with severe acute respiratory syndrome: a pathophysiological evaluation. Pathology 2006; 38: 210-218.

18. Bustamante Helfrich B, Chilukuri N, He H, *et al*. Maternal vascular malperfusion of the placental bed associated with hypertensive disorders in the Boston Birth Cohort. Placenta 2017; 52: 106-113 [published correction appears in Placenta 2019 Oct; 86: 52-53].

19. Khong TY, Mooney EE, Ariel I, *et al*. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Archives of Pathology and Laboratory Medicine 2016; 140: 698-713.

20. Alanjari A, Wright E, Keating S, *et al*. Prenatal diagnosis, clinical outcomes and associated pathology in pregnancies complicated by massive subchorionic thrombohematoma (Breus' mole). Prenatal Diagnosis, 2013;33:973-8.

21. Armstrong-Wells J, Donnelly M *et al*. Patterns of placental pathology in preterm premature rupture of membranes. Journal of Developmental Origins and Health Disease. 2013; 4(3): 249-255.

22. Ness RB, Bass D, Hill L, Klebanoff MA, Zhang J. Diagnostic test characteristics of placental weight in the prediction of small-for-gestational-age neonates. The Journal of Reproductive Medicine 2007; 52: 793-800.

23. McNamara H, Hutcheon JA, Platt RW, Benjamin A, Kramer MS. Risk factors for high and low placental weight. Paediatric and Perinatal Epidemiology 2014; 28: 97-105.

24. Redline RW, Boyd TK, Roberts DJ. Appendix 3 and 4. In Placental and gestational pathology. Cambridge University Press, 2019: 336-337.

Table 1. Clinical information and histomorphology of third trimester placentas from SARS-CoV-2-positive and SARS-CoV-2-negative women

			SARS-CoV-2 POSITIVE (N = 51)	SARS-CoV-2 NEGATIVE (N = 25)	p-value*
CLINICAL INFORMATION	Gestational Age	<37 weeks	10 (19.6%)	4 (16.0%)	1.00
		≥37 weeks	41 (80.4%)	21 (84.0%)	
	Comorbidities	Yes	21 (41.2%)	12 (48.0%)	0.63
		No	30 (58.8%)	13 (52.0%)	
	Delivery Method	Vaginal Delivery (VD)	26 (51.0%)	10 (40.0%)	0.47
		C-Section Delivery (CS)	25 (49.0%)	15 (60.0%)	
PATHOLOGIC INFORMATION	Ascending Intrauterine infection (AII)	Maternal Response	17 (33.3%)	9 (36.0%)	1.00
		Fetal Response	9 (17.7%)	3 (12.0%)	0.74
	Maternal Vascular Malperfusion (MVM)	DVA	3 (5.9%)	1 (4.0%)	1.00
		ACCVM/DVH	10 (19.6%)	1 (4.0%)	0.09
		VAG	21 (41.2%)	2 (8.0%)	**0.003**
		INF	7 (13.7%)	6 (24.0%)	0.33
		IVT	8 (15.7%)	7 (28%)	0.23
		SCT	9 (17.7%)	0	**0.026**
	Fetal Vascular Malformation (FVM)	AVASCS	5 (9.8%)	0	0.16
		FTV	4 (7.8%)	0	0.30
		CHORS	8 (15.7%)	2 (8.0%)	0.48
	Chronic villitis, unknown	CVUE	2 (3.9%)	2 (8.0%)	0.59

	etiology				

* p-value based on Fisher's exact test

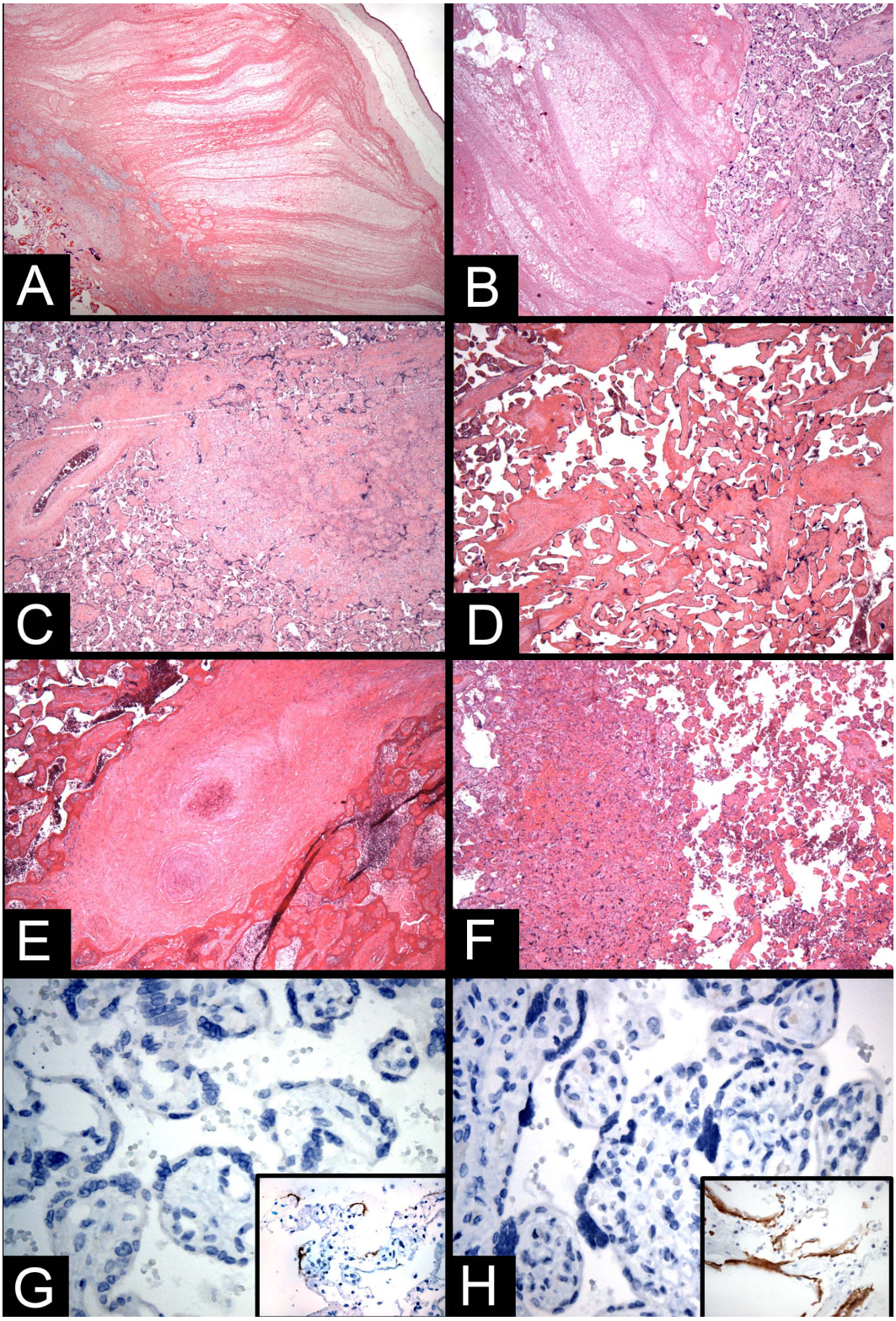
Abbreviations: AII: Ascending intrauterine infection; DVA: Decidual vasculopathy; ACCVM: Accelerated villous maturity; DVH: Distal villous hypoplasia; VAG: Villous agglutination; IVT: Intervillous thrombus; SCT: Subchorionic thrombus; INF: Infarct; AVASCS: Avascular villi, segmental; FTV: Fetal thrombotic vasculopathy; CHORS: Chorangiosis; CVUE: Chronic villitis, unknown etiology

Table 2. Clinical information and histomorphology of third trimester placentas from symptomatic and asymptomatic SARS-CoV-2-positive women

			SARS-CoV-2 POSITIVE		p-value*
			Symptomatic (N=25)	Asymptomatic (N=26)	
CLINICAL INFORMATION	Gestational Age	<37 weeks	6 (24%)	4 (15%)	0.50
		≥37 weeks	19 (76%)	22 (85%)	
	Comorbidities	Yes	12 (48%)	9 (35%)	0.40
		No	13 (52%)	17 (65%)	
	Delivery Method	Vaginal Delivery (VD)	12 (48%)	14 (54%)	0.78
		C-Section Delivery (CS)	13 (52%)	12 (46%)	
PATHOLOGIC INFORMATION	Ascending Intrauterine infection (AII)	Maternal Response	7 (28%)	10 (38%)	0.56
		Fetal Response	2 (8%)	7 (27%)	0.14
	Maternal Vascular Malperfusion (MVM)	DVA	1 (4%)	2 (7%)	1.00
		ACCVM/DVH	4 (16%)	6 (23%)	0.73
		VAG	12 (48%)	9 (35%)	0.40
		INF	3 (12%)	4 (15%)	1.00
		IVT	2 (8%)	6 (23%)	0.25
		SCT	7 (28%)	2 (8%)	0.07
	Fetal Vascular Malformation (FVM)	AVASCS	0	5 (19%)	0.051
		FTV	0	4 (15%)	0.11
		CHORS	3 (12%)	5 (19%)	0.70
	Chronic	CVUE	1 (4%)	1 (4%)	1.00

	villitis, unknown etiology				

Abbreviations: AII: Ascending intrauterine infection; DVA: Decidual vasculopathy; ACCVM: Accelerated villous maturity; DVH: Distal villous hypoplasia; VAG: Villous agglutination; IVT: Intervillous thrombus; SCT: Subchorionic thrombus; INF: Infarct; AVASCS: Avascular villi, segmental; FTV: Fetal thrombotic vasculopathy; CHORS: Chorangiosis; CVUE: Chronic villitis, unknown etiology



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