Mod Pathol 38 (2025) 100808

MODERN PATHOLOGY

USCAP UNITED STATES AND CANADIAN ACADEMY OF PATHOLOGY
Advancing Pathology Together

Journal homepage: https://modernpathology.org/

Research Article

The Spectrum of Placental Findings of First-Trimester Cytomegalovirus Infection Related to the Presence of Symptoms in the Newborns and Stillbirths

Eva Manuela Pena-Burgos[a,*], Rita María Regojo-Zapata[b], Ángela Caballero-Ferrero[c], Cristina Martínez-Payo[c], María del Carmen Viñuela-Benéitez[c], Dolores Montero[d], María De La Calle[e]

[a] *Department of Pathology, Gregorio Marañón University Hospital, Madrid, Spain;* [b] *Department of Pathology, La Paz University Hospital, Madrid, Spain;* [c] *Department of Obstetrics and Gynaecology, Gregorio Marañón University Hospital, Madrid, Spain;* [d] *Department of Microbiology, La Paz University Hospital, Madrid, Spain;* [e] *Department of Obstetrics and Gynaecology, La Paz University Hospital, Madrid, Spain*

ARTICLE INFO

Article history:
Received 16 February 2025
Revised 2 May 2025
Accepted 23 May 2025
Available online 6 June 2025

Keywords:
congenital cytomegalovirus
cytomegalovirus
cytomegalovirus villitis
placental study
villitis

ABSTRACT

Cytomegalovirus (CMV) is one of the most common congenital infections worldwide and a leading cause of prenatal neurological disorders, sensorineural hearing loss, and stillbirth. The placental factors involved in CMV transmission from mother to fetus remain poorly understood. We aimed to evaluate the histopathological placental findings associated with first-trimester CMV infection in relation to stillbirth and symptomatic presentation in newborns. This retrospective case-control study analyzed pregnancies with confirmed first-trimester CMV infection followed at 2 tertiary referral hospitals between 2012 and 2024. Symptomatic newborns were compared with asymptomatic newborns and stillbirths. Univariate statistical analyses were performed. A total of 40 placentas were included: 23 from asymptomatic newborns, 11 from symptomatic newborns, and 6 from stillbirths. Compared with asymptomatic cases, placentas from symptomatic newborns were smaller and showed increased chronic plasma cell deciduitis, chronic villitis (without avascular villi, breakdown villi, necrosis, or hemosiderin deposits), more CMV inclusions in fibroblasts, and higher rates of positive CMV immunostaining. Stillbirth placentas exhibited more severe chronic villitis (with avascular villi, breakdown villi, necrosis, and hemosiderin deposits), more extensive intervillous fibrin deposition, CMV inclusions in endothelial and trophoblastic cells, and higher frequencies of maternal and fetal vascular malperfusion compared with placentas from symptomatic newborns. Ultrasound screening did not appear to be a reliable predictor of placental involvement in first-trimester CMV infection. Greater involvement of the villous and vascular barriers—both direct and indirect—was associated with more severe fetal outcomes, likely facilitating viral transmission to the fetus. Adjunctive treatments aimed at reducing villous and vascular damage may help prevent symptomatic infection and stillbirth in first-trimester CMV cases.

Introduction

Cytomegalovirus (CMV) is one of the most common congenital infections worldwide and is associated with sensorineural hearing

* Corresponding author.
E-mail address: evapenaburgos.ep@gmail.com (E.M. Pena-Burgos).

0893-3952/$ - see front matter © 2025 United States & Canadian Academy of Pathology. Published by Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.
https://doi.org/10.1016/j.modpat.2025.100808

loss, neurological deficits, and retinopathy in newborns.[1,2] The global prevalence of congenital CMV is estimated to be between 0.2% and 2%.[3,4] Approximately 10% to 15% of affected newborns present with symptoms at birth, and an additional 10% to 15% of initially asymptomatic infants develop permanent sequelae—such as intellectual disability or hearing loss—over time.[1,4,5] First-trimester infection is linked to a higher risk of symptomatic disease during pregnancy and after birth.[3]

CMV is primarily transmitted from mother to fetus through the placenta, although rare cases of ascending genital tract infection have been reported.[6] Reactivation of latent CMV in maternal tissues can also result in fetal infection.[6] The maternal innate (natural killer cells and interleukins) and adaptive immune responses ($CD4^+$ and $CD8^+$ T cells) play a central role in controlling CMV infection.[7] Viral immune evasion mechanisms and the immunopathogenesis of CMV in pregnancy are still being investigated.[8] Administration of hyperimmune globulin has been shown to suppress viral replication and prevent placental dysfunction,[9,10] whereas valaciclovir therapy may reduce vertical transmission.[3]

Prenatal ultrasound findings related to fetal CMV infection are well documented.[5,11-14] Placental involvement can precede fetal anomalies.[5] However, ultrasound signs of CMV placental disease—such as placentomegaly, heterogeneous texture, or calcifications—are nonspecific and have not been thoroughly studied.[5,12,14] Gross and histopathological alterations in CMV-infected placentas have been reported, potentially interfering with placental development and function.[15-17] The specific placental factors contributing to fetal CMV transmission remain unclear.[8] The aim of this study was to analyze placental alterations associated with symptomatic congenital CMV infection and stillbirth following first-trimester maternal infection.

Materials and Methods

This retrospective case-control study compared symptomatic newborns (defined as the presence of at least 1 neurological or nonneurological CMV-related symptom within the first month of life, including neurodevelopmental delay, microcephaly, retinopathy, sensorineural hearing loss, or hepatosplenomegaly/hepatitis) with asymptomatic newborns and stillbirths (from 20-36 weeks of gestation) with congenital CMV infection. Only cases whose placentas were submitted to the Pathology Department between January 2012 and September 2024 were included.

Routine first-trimester blood screening was performed in all pregnant women around week 9. Immunoglobulin (Ig) G and IgM levels were measured. A positive IgM and low IgG avidity (<40%) indicated a primary CMV infection during pregnancy, whereas a high IgG avidity (≥40%) indicated a nonprimary infection.[5,8,13] Amniocentesis was recommended in these women; cases without amniocentesis were excluded. Inclusion criteria were based on a positive PCR result for CMV in the amniotic fluid. This was confirmed by a positive CMV PCR in neonatal urine or in amniotic fluid before delivery. In stillbirths, the infection was confirmed via positive amniotic fluid PCR. Fetal growth restriction (FGR), defined as less than 3rd percentile or <10th percentile with abnormal Doppler findings, was not considered a CMV-related symptom due to its multifactorial etiology. Elective terminations of pregnancy and infections diagnosed in the second or third trimesters were excluded.

Patient data were obtained from the Maternal-Fetal Medicine Units of 2 tertiary referral hospitals, following approval from the Institutional Research Ethics Committee (PI-5931 2023.793, approved on November 16, 2023). Informed consent was not required due to the retrospective observational nature of the study. All variables and definitions are summarized in the Supplementary Table S1.

Clinical information was retrieved from medical records, including maternal age, parity, preexisting maternal conditions, immunosuppression, gestational age at infection, treatment (CMV hyperimmune globulin ± valaciclovir), gestation type (singleton or multiple), FGR, premature rupture of membranes (PROMs), gestational age at delivery, mode of delivery (spontaneous, instrumental, or cesarean section), birthweight, 5-minute Apgar score, umbilical cord pH, neonatal symptoms, and symptoms at 6 months (such as mental retardation or hearing loss). In twin pregnancies, each fetus was analyzed individually. Antiviral treatment was initiated after maternal serological diagnosis and continued postamniocentesis until delivery.

Ultrasound evaluations of the fetus and placenta were performed using the GE Voluson E10 (GE Medical Systems) and Samsung HERAW 10 systems. Parameters were selected based on previously published criteria.[5,14,18] Brain abnormalities—including periventricular calcifications, microcephaly (≤2 SDs), periventricular or subependymal cysts, hemorrhagic areas, agenesis/dysgenesis of the corpus callosum, cerebellar hypoplasia, and ventriculomegaly (>10 mm)—were assessed. Cardiomegaly, pericardial effusion, hyperechogenic bowel, hepatomegaly, hepatic calcifications, and biliary lithiasis were also evaluated. These findings were grouped, and placental abnormalities such as placentomegaly, calcifications, and echogenic heterogeneity were assessed individually.

Gross placental data were obtained from pathology reports following the Amsterdam consensus and its updates.[19,20] Parameters included placental weight, maximum diameter, maximum thickness, abnormal cord insertion (marginal or velamentous), cord length, thickness, abnormal coiling (hypocoiling <1/10 cm or hypercoiling >8/10 cm), membrane discoloration (yellow or green), and hematomas (intraparenchymal, retroplacental, or subamniotic). Due to differences in gestational age, cord, and placental measurements were not compared between symptomatic newborns and stillbirths.

Histological assessment included hematoxylin and eosin–stained slides from umbilical cord, membranes, decidua, cord insertions, and villous parenchyma (2 central and 1 peripheral). Tissues were fixed in 10% formalin (24-48 hours), paraffin-embedded, and sectioned at 5 μm. CMV immunohistochemistry (IHC; clone CCH2 + DDG9, Ready to Use; Agilent-Dako) was reviewed. Two experienced placental pathologists (E.M.P.B., R.M.R.Z.) evaluated all cases blinded to neonatal outcomes, and diagnoses were determined by consensus. Histopathological diagnoses followed the Amsterdam criteria and updates.[19,20] Histological features were grouped into inflammatory (acute chorioamnionitis [grade, stage], acute funisitis [grade, stage], chronic villitis [grade, stage, and cellular type], chronic intervillositis, and plasma cell deciduitis), vascular (accelerated villous maturation, distal villous hypoplasia, avascular villi unrelated to villitis, stem vessel obliteration, stromal-vascular karyorrhexis, and fetal thrombotic vasculopathy), and other patterns (edema, intervillous fibrin not related to villitis, dystrophic calcifications, and amniotic membrane alterations). Amniotic alterations included columnar change (≥30% of epithelial cells), cell fusion (pseudostratified epithelium in ≥30%), and blebbing (cytoplasmic vacuolation in ≥30%). CMV inclusions were assessed by hematoxylin and eosin and IHC and considered positive if at least 1 infected cell (fibroblast, endothelial cell, trophoblast, or macrophage) was identified. Chronic villitis-associated features

(avascular villi, trophoblastic basement membrane breakdown ≥30%, villous necrosis ≥10%, and hemosiderin-laden macrophages) were recorded. Placentas with no macroscopic or microscopic abnormalities and negative CMV IHC were considered "normal." Fetal autopsies were performed in all stillbirths, and CMV inclusions were sought in fetal organs. Viral load in placental tissue was not routinely assessed.

Quantitative variables were expressed as means ± standard deviation (SD) for normally distributed data or medians and interquartile ranges for nonnormally distributed data. Qualitative variables were described as frequencies and percentages. The Kolmogorov-Smirnov and Shapiro-Wilk tests assessed normality. Qualitative comparisons were performed using χ^2 or Fisher exact tests, and quantitative comparisons used Student *t* test (parametric) or Mann-Whitney *U* test (nonparametric). Associations for qualitative variables were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A 2-tailed $P < .05$ was considered statistically significant. Statistical analysis was conducted using SPSS software, version 25 (SPSS, Inc).

Results

A total of 133,692 births occurred between January 2012 and September 2024 in the 2 participating hospitals, with 13,650 placentas (10.21%) submitted to the Pathology Department for analysis. There were 2678 stillbirths (1.76%), and autopsy studies (including placental evaluation) were requested in 2366 cases (88.34%).

We initially reviewed 168 pregnant women with positive first-trimester anti-CMV IgM and IgG serologies. Amniocentesis was performed in 145 patients, and 82 were positive for CMV on PCR testing. Of these, 3 opted for elective legal termination (at 20-21 weeks of gestation), and 39 placentas were not sent for pathological evaluation. Ultimately, 40 patients were included in this study: 6 had stillbirths, 11 had symptomatic newborns, and 23 had asymptomatic newborns (Fig. 1). Key findings comparing symptomatic and asymptomatic newborns are summarized in Tables 1 and 2; comparisons with stillbirths are summarized in Tables 3 and 4. Statistically significant findings and others not included in the tables are highlighted below.

The most frequent maternal comorbidities were prepregnancy hypothyroidism ($n = 6$), gynecologic disorders ($n = 4$), and prepregnancy diabetes ($n = 2$). The most common ultrasound abnormalities involved the central nervous system ($n = 16$), the heart ($n = 3$), and the intestine ($n = 2$) (Fig. 2). Among multiple pregnancies, 2 were dichorionic diamniotic and 1 was monochorionic diamniotic.

Twenty-nine women (85.3%) received treatment during pregnancy—22 (95.7%) in the asymptomatic group and 7 (63.6%) in

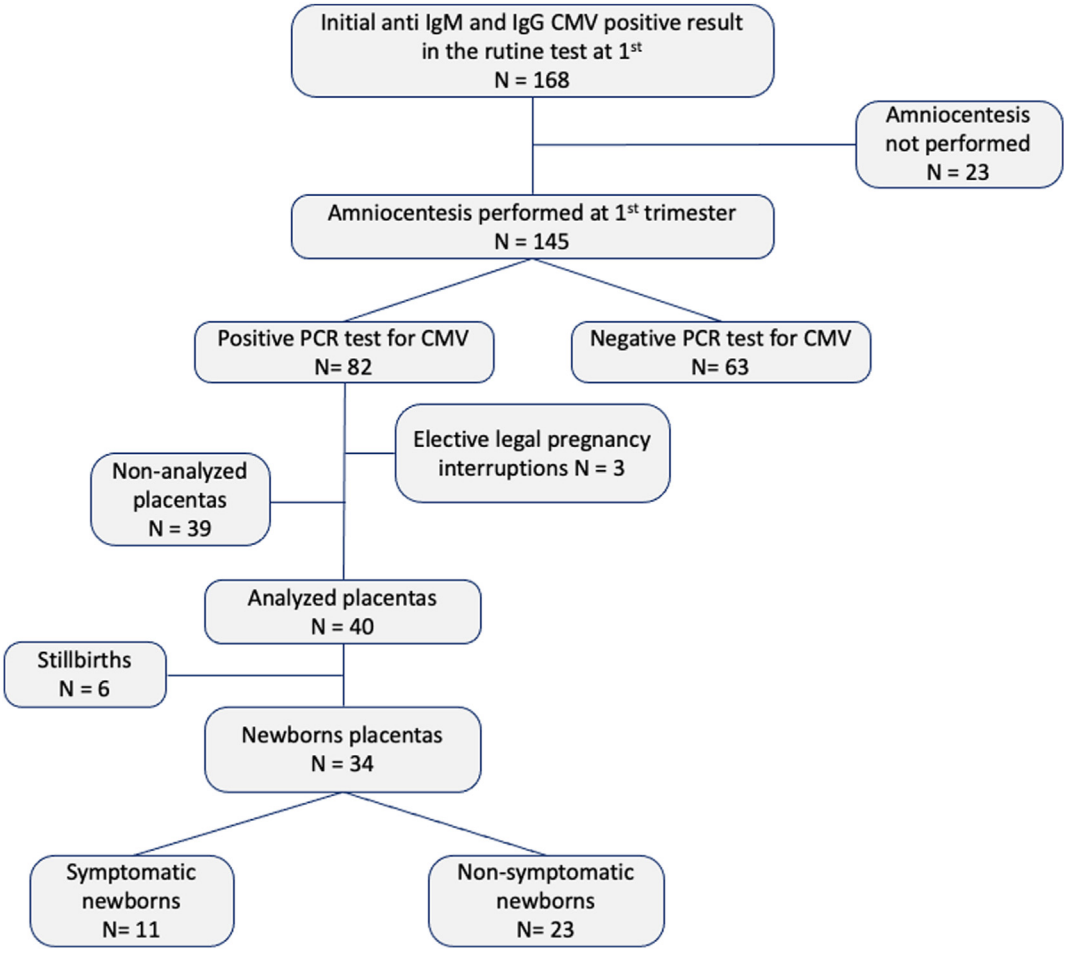

Figure 1.
Inclusion and exclusion criteria. Number of placentas analyzed. CMV, cytomegalovirus.

Table 1
Description of maternal, obstetric, neonatal and cytomegalovirus infection variables, statistical significance, and factors associated with symptomatic newborns

Variable	Total, $n = 34$	Symptomatic, $n = 11$	Asymptomatic, $n = 23$	Significance (P)	Odds ratio (OR)	95%CI
Maternal characteristics						
Maternal age (y)	33 (P25 = 32; P75 = 37)	33 (P25 = 31; P75 = 37)	33 (P25 = 32; P75 = 37)	.92[a]	–	–
Primiparous	9 (26.5)	2 (18.2)	7 (30.4)	.68[b]	1.96	0.–11.57
Assisted reproductive technology	4 (11.8)	1 (9.1)	3 (13.0)	1.00[b]	0.66	0.06–7.25
Maternal disease	18 (52.9)	5 (45.5)	13 (56.5)	.54[b]	1.56	0.36–6.61
CMV infection data						
Weeks of detection	13 (P25 = 10; P75 = 24)	12 (P25 = 10; P75 = 22)	13 (P25 = 11; P75 = 24)	.78[a]	–	–
Maternal serology				.70[b]	1.71	0.35–8.23
Primary infection	22 (64.7)	8 (72.7)	14 (60.9)			
Reactivation	12 (35.3)	3 (27.3)	9 (39.1)			
Treatment administered	29 (85.3)	7 (63.6)	22 (95.7)	.02[b]	0.08	0.01–0.83
Type of treatment				.68[b]	1.60	0.28–8.90
Hyperimmune globulin + Valaciclovir	14 (48.3)	4 (57.1)	10 (45.5)			
Hyperimmune globulin	15 (51.7)	3 (42.9)	12 (41.4)			
Obstetric and fetal characteristics						
Pregnancy type (fetus)				.05[b]	0.59	0.43–0.81
Single	27	11 (100)	16 (72.7)			
Multiple	6	0 (0)	6 (27.3)			
Premature ruptura of membranes	3 (8.8)	0 (0)	3 (13.0)	.53[b]	0.64	0.49–0.83
Fetal growth restriction	6 (17.6)	5 (45.5)	1 (4.3)	<.01[b]	18.33	1.78–45.92
Birth weeks	37 (P25 = 36; P75 = 39)	35 (P25 = 35; P75 = 40)	37 (P25 = 35; P75 = 39)	.96[a]	–	–
Noneuthocic delivery	12 (37.5)	5 (45.5)	7 (33.3)	.50[b]	1.66	0.37–7.42
Ultrasound findings						
Fetal ultrasound alterations	14 (41.2)	9 (81.8)	5 (21.7)	<.01[b]	16.20	8.61–23.21
Placental ultrasound alterations						
Placentomegaly	4 (11.8)	2 (18.2)	2 (8.7)	.42[b]	2.33	0.28–19.24
Calcifications	0 (0)	0 (0)	0 (0)	–	–	–
Echogenic heterogeneity	0 (0)	0 (0)	0 (0)	–	–	
Neonatal outcomes						
Birth weight (grams)	2609.00 (P25 = 2238.75; P75 = 3112.50)	2562.03 (P25 = 2142.43; P75 = 3023.02)	2747.20 (P25 = 2331.37; P75 = 3277.89)	.61[a]	–	–
Apgar test (5 min)	9 (P25 = 8; P75 = 9)	9 (P25 = 8; P75 = 9)	9 (P25 = 8; P75 = 9)	.53[a]	–	–
Umbilical cord pH	7.31 (P25 = 7.21, P75 = 7.35)	7.28 (P25 = 7.19, P75 = 7.32)	7.32 (P25 = 7.23, P75 = 7.35)	.46[a]	–	–
Neonatal death	1 (3.1)	1 (9.1)	0 (0)	.16[b]	0.32	0.14–0.78
Long-term outcomes						
Long-term sequelae	5 (14.7)	5 (45.5)	0 (0)	<.01[b]	0.20	0.10–0.42

CMV, cytomegalovirus.
Kolmogorov-Smirnov test P results: maternal age: $P < 0.01$; weeks of CMV infection detection: $P = 0.03$; birth weeks: $P = 0.02$; birth weight: $P = 0.04$, Apgar test: $P < 0.01$; umbilical cord pH: $P < 0.01$.
[a] Data compared with Mann-Whitney U test.
[b] Data compared with Fisher exact test.

Table 2
Description of gross and histological placental variables, statistical significance, and factors associated with symptomatic newborns

Variable	Total, $n = 34$	Symptomatic, $n = 11$	Asymptomatic, $n = 23$	Significance (P)	Odds ratio (OR)	95%CI
Gross characteristics						
Placental weight (grams)	477.79 ± 143.52	398.91 ± 108.76	519.11 ± 144.22	.02[a]	–	–
<10th percentile	9 (26.5)	6 (54.5)	3 (13.0)	.03[b]	8.00	1.46-23.67
>90th percentile	4 (11.8)	2 (18.2)	2 (8.7)	.58[b]	2.33	0.28-19.24
Placental size (cm, maximum diameter)	17.75 ± 4.12	15.54 ± 1.91	18.95 ± 4.48	.02[a]	–	–
Placental thickness (cm)	2.57 ± 0.50	2.20 ± 0.33	2.75 ± 0.47	<.01[a]	–	–
Abnormal umbilical cord insertion (marginal/ velamentous)	3 (8.8)	1 (9.1)	2 (8.7)	.97[b]	1.05	0.08-12.99
Umbilical cord length	29.82 ± 14.92	28.40 ± 16.86	30.57 ± 14.18	.70[a]	–	–
Umbilical cord diameter	1.21 ± 0.26	1.13 ± 0.21	1.25 ± 0.28	.23[a]	–	–
Abnormal umbilical cord coiling	4 (12.1)	1 (10.0)	3 (13.0)	.80[b]	0.74	0.06-8.13
Membranous alterations	11 (33.3)	3 (30.0)	8 (34.8)	.78[b]	0.80	0.16-3.98
Intraparenchymatous infarcts	7 (20.6)	3 (27.3)	4 (17.4)	.50[b]	1.78	0.32-9.84
Subchorionic hematomas	3 (9.4)	1 (9.1)	2 (9.5)	.96[b]	0.95	0.07-11.80
Histopathological characteristics						
Inflammatory pattern findings						
Acute chorioamnionitis	15 (45.5)	5 (45.5)	10 (43.5)	.81[b]	1.20	0.26-5.36
Acute funisitis	6 (17.6)	0 (0)	6 (26.1)	.06[b]	0.60	0.45-0.81
Chronic plasma cell deciduitis	2 (6.3)	2 (18.2)	0 (0)	.04[b]	0.30	0.17-0.51
Chronic villitis	8 (25.0)	7 (63.6)	1 (4.8)	<.01[b]	35.00	10.3-51.43
Avascular villi	1 (3.1)	1 (9.1)	0 (0)	.16[b]	0.32	0.19-0.53
Villi necrosis	1 (3.1)	1 (9.1)	0 (0)	.16[b]	0.32	0.19-0.53
Villi breakdown	0 (0)	0 (0)	0 (0)	–	–	–
Hemosiderin villi deposition	0 (0)	0 (0)	0 (0)	–	–	–
Chronic villitis grade	2 (25.0)	1 (50.0)	1 (50.0)	.25[b]	0.50	0.12-1.99
Low grade	1 (50.0)	0 (0)	1 (100)	1.00[b]	–	–
Focal	1 (50.0)	1 (100)	0 (0)			
Multifocal	6 (75.0)	6 (100)	0 (0)			
High grade	4 (66.6)	4 (66.6)	0 (0)	1.00[b]	–	–
Patchy	2 (33.3)	2 (33.3)	0 (0)			
Diffuse						
Chronic intervillositis	1 (3.1)	1 (9.1)	0 (0)	.16[b]	0.32	0.19-0.53
Vascular pattern findings						
Accelerated villous maturation or distal villous hypoplasia	4 (18.2)	0 (0)	4 (12.5)	.28[b]	0.64	0.48-0.84
Stromal-vascular karyorrhexis or avascular villi nonrelated to villitis	0 (0)	0 (0)	0 (0)	–	–	–
Fetal thrombotic vasculopathy	0 (0)	0 (0)	0 (0)	–	–	–
Other findings						
Membranes' changes						
Columnar changes	1 (3.1)	1 (9.1)	0 (0)	.16[b]	0.32	0.19-0.53
Cell fusion	1 (3.1)	1 (9.1)	0 (0)	.16[b]	0.32	0.19-0.53
Blebbing	1 (3.1)	1 (9.1)	0 (0)	.16[b]	0.32	0.19-0.53
Villous edema	2 (6.3)	1 (9.1)	1 (4.8)	.52[b]	2.44	0.13-43.47
Chorangiosis	3 (9.4)	1 (9.1)	2 (9.5)	.90[b]	1.16	0.09-14.56
Dystrophic calcifications	23 (69.7)	8 (80.0)	15 (65.2)	.68[b]	2.13	0.36-12.54
Intervillous fibrin deposits	5 (15.2)	3 (30.0)	2 (8.7)	.14[b]	4.50	0.61-32.69
Nucleated red blood cells	2 (6.3)	0 (0)	2 (9.5)	1.00[b]	0.67	0.53-0.86
Viral CMV inclusions	4 (11.8)	4 (36.4)	0 (0)	<.01[b]	0.23	0.12-0.44
Fibroblasts	4 (11.8)	4 (36.4)	0 (0)	<.01[b]	0.23	0.12-0.44
Macrophages	1 (3.1)	1 (9.1)	0 (0)	.16[b]	0.32	0.19-0.53
Endothelial cells	0 (0)	0 (0)	0 (0)	–	–	–
Trophoblast	0 (0)	0 (0)	0 (0)	–	–	–
Immunohistochemical results						
Positive immunostaining for CMV	6 (17.6)	6 (54.5)	0 (0)	<.01[b]	0.17	0.08-0.39
"Normal" placentas	5 (14.7)	0 (0)	5 (21.7)	.15[b]	0.62	0.46-0.82

CMV, cytomegalovirus.
Kolmogorov-Smirnov test P results: placental weight: $P = .21$; placental size: $P = .34$; placental thickness: $P = .16$; umbilical cord length: $P = .54$; umbilical cord diameter: $P = .35$.
[a] Data compared with Student t test.
[b] Data compared with Fisher exact test.

Table 3
Description of maternal, obstetric, neonatal, and cytomegalovirus infection variables, statistical significance, and factors associated with stillbirths

Variable	Total, $n = 17$	Symptomatic, $n = 11$	Stillbirths, $n = 6$	Significance (P)	Odds ratio (OR)	95%CI
Maternal characteristics						
Maternal age (y)	37 (P25 = 33.5; P75 = 39.5)	33 (P25 = 31; P75 = 37)	39.5 (P25 = 37.7; P75 = 41.2)	.01[a]	–	–
Primiparous	5 (29.4)	2 (18.2)	3 (50.0)	.16[b]	0.22	0.02–2.03
Assisted reproductive technology	1 (5.9)	1 (9.1)	0 (0)	1.00[b]	0.62	0.42–0.91
Maternal disease	9 (52.9)	5 (45.5)	4 (66.6)	.85[b]	0.83	0.11–6.11
CMV infection data						
Weeks of detection	13 (P25 = 10.5; P75 = 14.5)	12 (P25 = 10; P75 = 22)	11 (P25 = 9.7; P75 = 12.25)	.14[a]	–	–
Maternal serology				.62[b]	1.87	0.15–23.39
Primary infection	13 (76.5)	8 (72.7)	5 (83.3)			
Reactivation	4 (23.5)	3 (27.3)	1 (16.7)			
Treatment administered	9 (52.9)	7 (63.6)	2 (33.3)	.23[b]	0.28	0.03–2.32
Type of treatment				.85[b]	0.75	0.03–17.50
Hyperimmune globulin + Valaciclovir	5 (55.6)	4 (57.1)	1 (16.7)			
Hyperimmune globulin	4 (44.4)	3 (42.9)	1 (16.7)			
Ultrasound findings						
Fetal ultrasound alterations	11 (64.7)	9 (81.8)	2 (33.3)	.10[b]	0.11	0.01–1.09
Placental ultrasound alterations						
Placentomegaly	3 (18.8)	2 (18.2)	1 (16.7)	.86[b]	0.36	0.16–0.86
Calcifications	0 (0)	0 (0)	0 (0)	–	–	–
Echogenic heterogeneity	0 (0)	0 (0)	0 (0)	–	–	–
Obstetric and fetal characteristics						
Premature rupture of membranes	0 (0)	0 (0)	0 (0)	–	–	–
Fetal growth restriction	10 (17.6)	5 (45.5)	5 (83.3)	.30[b]	6.00	0.51–23.75
Birth weeks	36 (P25 = 27; P75 = 39)	35 (P25 = 35; P75 = 40)	25 (P25 = 20; P75 = 33)	<.01[a]	–	–

CMV, cytomegalovirus.
Kolmogorov-Smirnov test P results: maternal age: $P < .01$; weeks of CMV infection detection: $P = .03$; birth weeks: $P = .02$.
[a] Data compared with Mann-Whitney U test.
[b] Data compared with Fisher exact test.

the symptomatic group—with a statistically significant difference ($P = .02$; OR, 0.08; 95% CI, 0.01-0.83). FGR developed in 6 cases (17.6%), 5 of which occurred in symptomatic newborns, with a significant difference ($P < .01$; OR, 18.33; 95% CI, 1.78-45.92). Among symptomatic neonates, sensorineural hearing loss was observed in 7 cases, neurodevelopmental delay in 6 cases, microcephaly in 3 cases, retinopathy in 2 cases, and hepatosplenomegaly in 2 cases. One neonatal death occurred at 2 days of life. Symptoms at 6 months were recorded in 6 symptomatic newborns ($P < .01$; OR, 0.20; 95% CI, 0.10-0.42), including sensorineural deafness and mental retardation ($n = 3$ each).

Median maternal age in the stillbirth group was 39.5 years (P25 = 37.7; P75 = 41.2), significantly higher than in symptomatic newborns ($P = .01$). Median gestational age at delivery was 25 weeks (P25 = 20; P75 = 33) in the stillbirth group ($P < .01$ vs symptomatic newborns).

The mean placental weight was 477.79 ± 143.52 g, with significant differences between groups ($P = .02$). Hypoplastic placentas (<10th percentile) were more common in the symptomatic group ($P = 0.03$; OR, 8.00; 95% CI, 1.46-23.67). Mean placental size was 17.75 ± 4.12 cm ($P = .02$), and thickness was 2.57 ± 0.50 cm ($P < .01$). The main histopathological findings in symptomatic newborns are shown in Figure 3 and in stillbirths in Figure 4.

Acute funisitis was only seen in the asymptomatic group ($n = 6$, grade 1/stage 1) ($P = .06$). Acute chorioamnionitis was generally grade 2 or 3, stage 2. Chronic plasma cell deciduitis was detected only in 2 symptomatic cases ($P = .04$; OR, 0.30; 95% CI, 0.17-0.51). Chronic lymphoplasmacytic villitis was found in 25.0% of all placentas and 63.6% of the symptomatic group ($P < .01$; OR, 35.00; 95% CI, 10.30-51.43). No differences were found by grade. Viral inclusions were observed in 4 (11.8%) symptomatic cases, and CMV immunostaining was positive in 6 (17.6%) ($P < .01$ for both comparisons). Inclusions were seen in fibroblasts ($n = 4$) and macrophages ($n = 1$). Five (21.7%) placentas in the asymptomatic group were entirely normal with negative CMV IHC.

Villitis with avascular villi ($P < .01$; OR, 7.00; 95% CI, 1.14-18.85), necrosis ($P < .01$; OR, 6.78; 95% CI, 1.56-17.65), villous breakdown ($P < .01$; OR, 6.78; 95% CI, 0.56-17.65), and hemosiderin deposition ($P = .01$; OR, 5.35; 95% CI, 1.32-15.47) were significantly more common in stillbirths than symptomatic newborns. Regarding vascular lesions, accelerated villous maturation, distal villous hypoplasia, avascular villi (not related to villitis), stromal-vascular karyorrhexis, and fetal thrombotic vasculopathy were more prevalent in stillbirths (all $P < .02$; OR, 0.21; 95% CI, 0.07-0.58). Intervillous fibrin ($P < .01$; OR, 6.57; 95% CI, 1.21-17.75), nucleated red blood cells ($P < .01$; OR, 0.15; 95% CI, 0.04-0.55), and membrane changes ($P = .01$; OR, 5.35; 95% CI, 1.32-15.47) were also more frequent in stillbirths.

Table 4
Description of gross and histological placental variables, statistical significance, and factors associated with stillbirths

Variable	Total, $n = 17$	Symptomatic, $n = 11$	Stillbirths, $n = 6$	Significance (P)	Odds ratio (OR)	95%CI
Gross characteristics						
Abnormal umbilical cord insertion (marginal/ velamentous)	1 (5.9)	1 (9.1)	0 (0)	.44[a]	0.62	0.42-12.99
Membranous alterations	6 (35.3)	3 (27.3)	3 (50.0)	.34[a]	2.66	0.33-21.32
Intraparenchymatous infarcts	5 (29.4)	3 (27.3)	2 (33.3)	.86[a]	1.33	0.15-11.49
Subchorionic hematomas	1 (5.9)	1 (9.1)	0 (0)	1.00[a]	0.62	0.42-0.91
Histopathological characteristics						
Inflammatory pattern findings						
Acute chorioamnionitis	7 (41.2)	5 (45.5)	2 (33.3)	.62[a]	0.60	0.76-4.76
Acute funisitis	0 (0)	0 (0)	0 (0)	–	–	–
Chronic deciduitis	4 (23.5)	2 (18.2)	2 (33.3)	.58[a]	2.25	0.22-15.14
Chronic villitis	13 (76.5)	7 (63.6)	6 (100)	.09[a]	1.85	1.12-3.07
Avascular villi	7 (41.2)	1 (9.1)	6 (100)	<.01[a]	7.00	1.14-18.85
Villi necrosis	6 (35.3)	1 (9.1)	5 (83.3)	<.01[a]	6.78	1.56-17.65
Villi breakdown	6 (35.5)	1 (9.1)	5 (83.3)	<.01[a]	6.78	1.56-17.65
Hemosiderin villi deposition	5 (29.4)	1 (9.1)	4 (66.7)	.01[a]	5.35	1.32-15.47
Chronic villitis grade						
Low grade	1 (7.7)	1 (100.0)	0 (0)	1.00[a]	2.00	1.13-3.52
Focal	0 (0)	0 (0)	0 (0)	1.00[a]	–	–
Multifocal	1 (100)	1 (100)	0 (0)			
High grade	12 (92.3)	6 (100)	6 (100)			
Patchy	7 (58.3)	4 (66.6)	3 (50.0)	1.00[a]	2.00	0.19-20.61
Diffuse	5 (38.4)	2 (33.3)	3 (50.0)			
Chronic intervillositis	1 (5.9)	1 (9.1)	0 (0)	.44[a]	0.62	0.42-0.91
Vascular pattern findings						
Accelerated villous maturation or distal villous hypoplasia	3 (16.6)	0 (0)	3 (50.0)	.02[a]	0.21	0.07-0.58
Stromal-vascular karyorrhexis or Avascular villi nonrelated to villitis	3 (17.6)	0 (0)	3 (50.0)	.02[a]	0.21	0.07-0.58
Fetal thrombotic vasculopathy	3 (17.6)	0 (0)	3 (50.0)	.02[a]	0.21	0.07-0.58
Other findings						
Membranes' changes						
Columnar changes	5 (29.4)	1 (9.1)	4 (66.7)	.01[a]	5.35	1.32-15.47
Cell fusion	5 (29.4)	1 (9.1)	4 (66.7)	.01[a]	5.35	1.32-15.47
Blebbing	5 (29.4)	1 (9.1)	4 (66.7)	.01[a]	5.35	1.32-15.47
Villous edema	1 (5.9)	1 (9.1)	0 (0)	.44[a]	0.62	0.42-0.19
Chorangiosis	1 (5.9)	1 (9.1)	0 (0)	.44[a]	0.62	0.42-0.19
Dystrophic calcifications	12 (70.6)	8 (72.2)	4 (66.7)	.79[a]	0.75	0.08-6.46
Intervillous fibrin deposits	9 (52.9)	3 (27.3)	6 (100)	<.01[a]	6.57	1.21-17.75
Nucleated red blood cells	4 (23.5)	0 (0)	4 (66.7)	<.01[a]	0.15	0.04-0.55
Viral CMV inclusions	10 (58.8)	4 (36.4)	6 (100)	.03[a]	2.50	1.17-5.34
Fibroblasts	8 (47.1)	4 (36.4)	4 (66.7)	.23[a]	3.50	0.43-28.44
Macrophages	2 (11.8)	1 (9.1)	1 (16.6)	.64[a]	2.00	0.10-39.07
Endothelial cells	4 (23.5)	0 (0)	4 (66.7)	<.01[a]	0.15	0.04-0.55
Trophoblast	2 (11.8)	0 (0)	2 (33.3)	.04[a]	0.26	0.11-0.61
Immunohistochemical results						
Positive immunostaining for CMV	12 (70.6)	6 (54.5)	6 (100)	.04[a]	2.00	1.13-0.39

CMV, cytomegalovirus.
[a] Data compared with Fisher exact test.

Viral inclusions ($P = .03$; OR, 2.50; 95% CI, 1.17-5.34) and positive immunostaining ($P = 0.04$; OR, 2.00; 95% CI, 1.13-4.39) were found in all stillbirth placentas. Inclusions were more frequently found in endothelial cells ($P < .01$; OR, 0.15; 95% CI, 0.04-0.55) and trophoblasts ($P = .04$; OR, 0.26; 95% CI, 0.11-0.61). All fetal autopsies revealed multiorgan CMV inclusions, with no or minimal tissue autolysis.

Discussion

In summary, chronic lymphoplasmacytic villitis and chronic deciduitis were more frequently observed in symptomatic newborns than in asymptomatic ones. The presence of chronic lymphoplasmacytic villitis associated with avascular villi, villous breakdown, necrosis, or hemosiderin deposits, as well as chronic

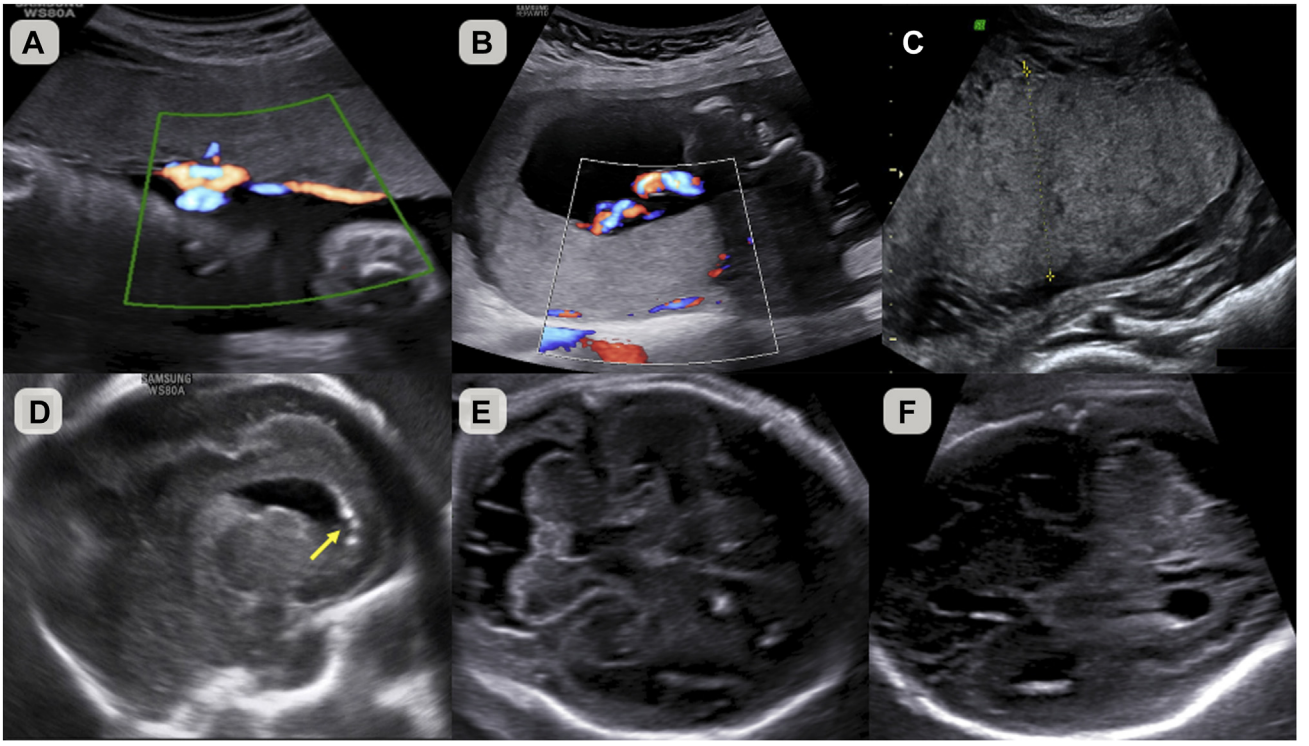

Figure 2.
Ultrasound findings of placentas and fetuses. (A) 30 Weeks normal placental ultrasound of a symptomatic newborn; (B) 30 weeks normal placental ultrasound of a stillbirth; (C) 20 weeks placentomegaly (arrow) of a symptomatic newborn; (D) periventricular calcifications (arrow); (E) cerebellous hypoplasia; (F) polymicrogyria and anomalous Sylvian fissure. (D–F) are the same case as placenta (B).

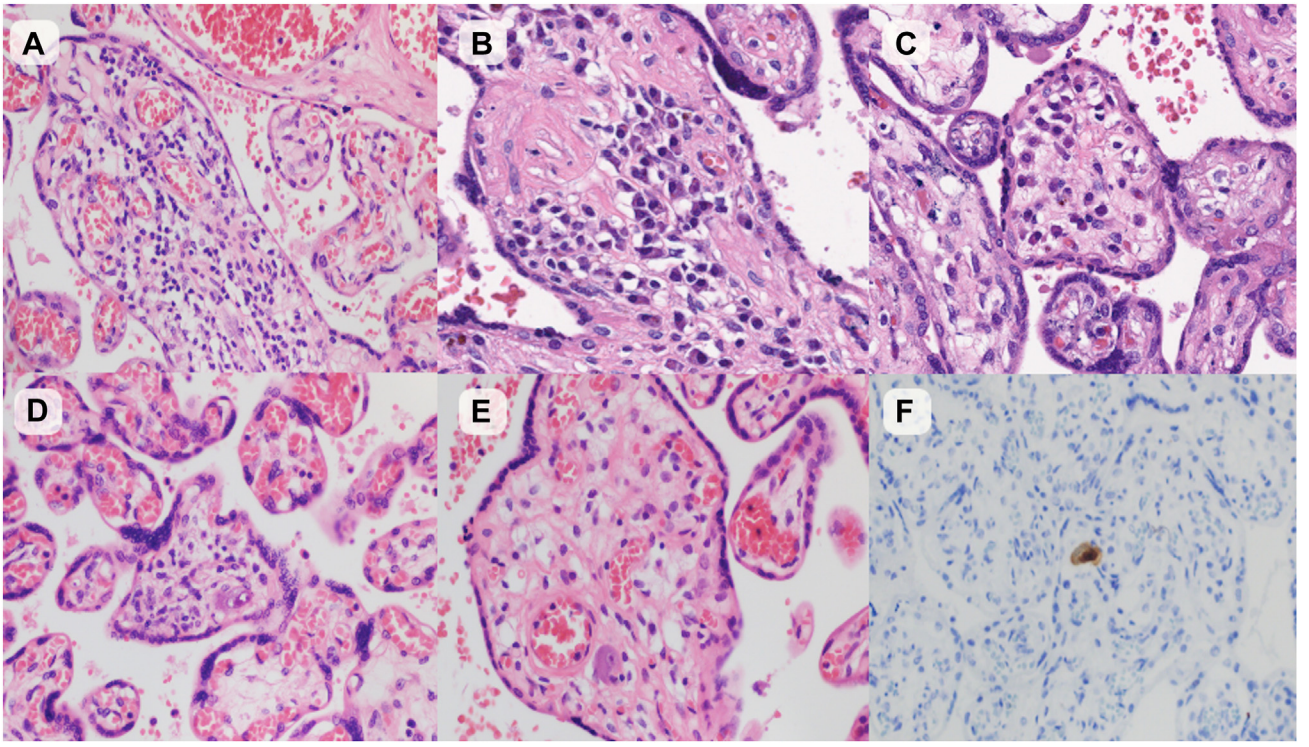

Figure 3.
Histopathological findings of symptomatic newborn placentas. (A) (H and E, ×100), (B, C) (H and E, ×200): lymphoplasmacytic villitis without architectural villi distortion; (D, E) (H and E, ×400): CMV viral inclusions in fibroblastic cells; (F) (CMV, ×200): CMV-infected positive cell. CMV, cytomegalovirus; H and E, hematoxylin and eosin.

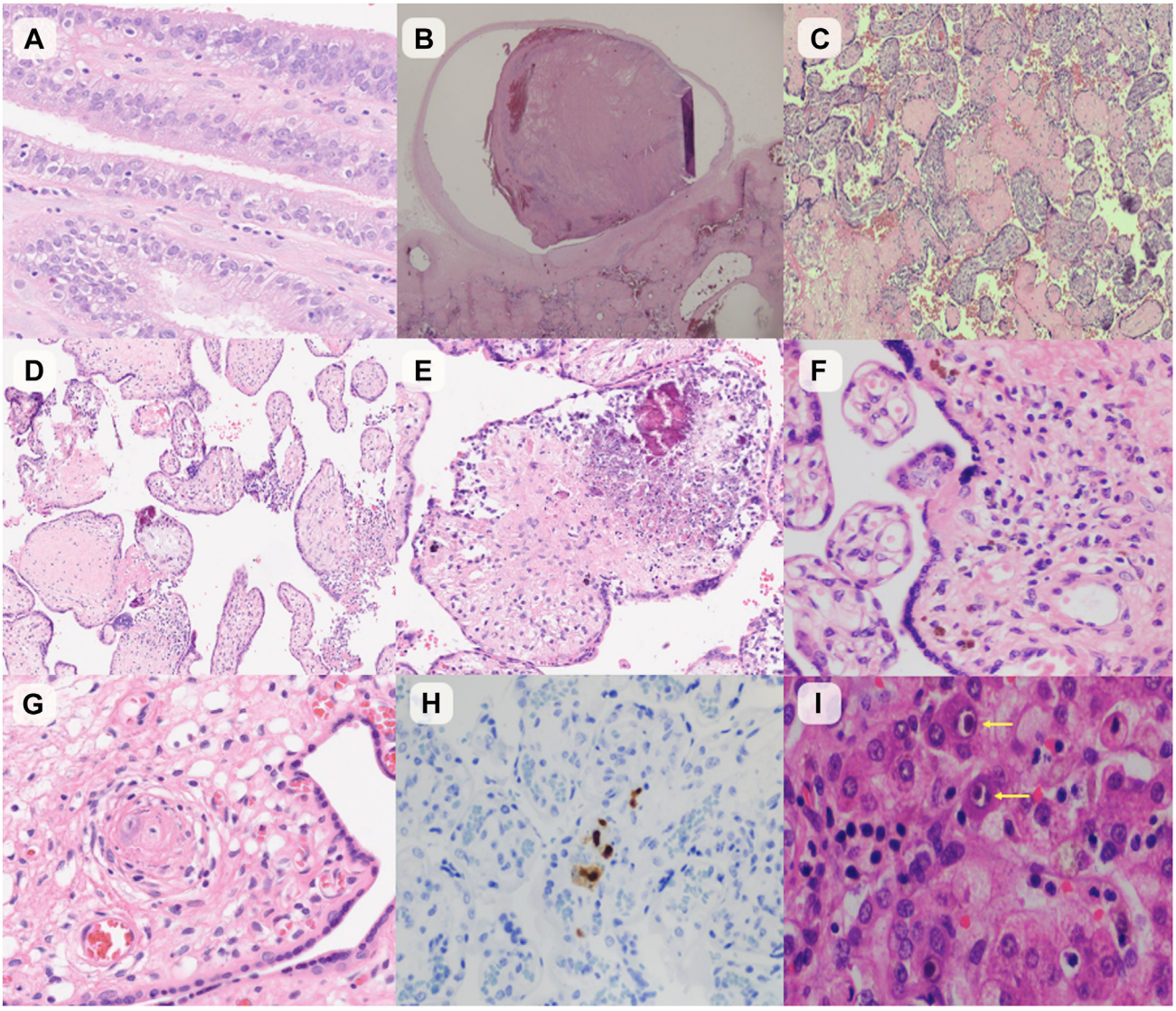

Figure 4.
Histopathological findings of stillbirth placentas and fetuses. (A) (H and E, ×400): Membranous changes (blebbing, columnar cells, pseudostratification) (B) (H and E, ×40): Organized trombi in a big chorionic vessel; (C) (H&E, ×40), (D) (H&E, ×100), (E) (H and E, ×200): lymphoplasmacytic villitis with fibrin deposition, dystrophic calcifications, avascular villi, necrosis and villous breakdown; (F) (H and E, ×400): hemosiderin deposition in villous macrophages; (G) (H and E, ×400): CMV viral inclusion in endothelial cell with vessel obliteration; (H) (CMV, ×200): CMV-infected positive cells; (I) (H and E, ×400): liver CMV viral inclusions. CMV, cytomegalovirus; H and E, hematoxylin and eosin.

deciduitis, intervillous fibrin deposits, nucleated red blood cells, and maternal and fetal malperfusion patterns, was more frequently associated with stillbirth.

In this study, 40 placentas from fetuses with congenital CMV infection were analyzed. To our knowledge, only 1 previously published study has compared findings in symptomatic and asymptomatic newborns infected with congenital CMV, including 35 placentas.[8] Advanced maternal age was present in the stillbirth cases, a factor generally associated with poorer pregnancy outcomes[21] and possibly contributing to impaired immune response to CMV. No differences were found in parity, antiretroviral therapy use, or the presence of previous diseases. A history of previous pregnancies could be a risk factor because transmission from young children is a main route of CMV infection.[2] The most frequently observed maternal condition was hypothyroidism, present in 25% of cases. A potential association between congenital CMV infection and hypothyroidism has been proposed in a few case reports.[22,23] However, hypothyroidism is common in high-risk pregnancies.

Most of our cases were primary infections, with no intergroup differences. Placentitis usually follows a primary infection, although reactivation may also cause significant placental involvement.[24] Some authors have reported higher rates of symptomatic newborns in nonprimary CMV infections,[25] whereas others have not observed differences.[8,26] In our series, infection was detected around week 12 in most cases due to routine first-trimester screening. Although vertical transmission is less likely during this stage, fetal involvement tends to be more severe because organogenesis is ongoing.[2] No correlation was found between the timing of detection (within the first trimester) and the occurrence of symptoms or stillbirth, although all symptomatic cases were first-trimester infections. Other factors likely play a role in fetal transmission.[6]

Treatment during pregnancy has been associated with fewer fetal complications and symptoms.[1,10,27] Valaciclovir (2 g every 6 hours) reduces vertical transmission, although its impact on fetal symptoms is still being studied.[1] Hyperimmune globulin (200 IU/kg) may be considered for very recent primary infections to prevent vertical transmission.[1,28] Uenaka et al[8] did not find histological differences between treated and untreated women.[8] In our study, 9 women received treatment, yet symptomatic newborns or stillbirths still occurred. New therapeutic approaches involving natural killer cells and T lymphocytes should be explored. Natural killer cells play a role in innate immunity, whereas T lymphocytes mediate adaptive immunity against CMV. CMV immune evasion mechanisms are still being studied.[1,3,7]

Ultrasonography and magnetic resonance imaging are the most common imaging modalities in CMV-infected pregnancies. At our centers, neurosonography is performed every 3 to 4 weeks, requiring high-resolution equipment and skilled obstetricians.[29] Magnetic resonance imaging is more sensitive for detecting third-trimester central nervous system abnormalities,[14] identifying up to 6% of anomalies in cases with normal neurosonography.[30] Ultrasound-detected fetal abnormalities have been linked to neonatal symptoms,[1] as in our study. Placental ultrasound abnormalities are found in only 4.3% of congenital CMV cases.[18] In our series, placentomegaly was present in 12.5% of cases, with no intergroup differences, and we did not observe calcifications or heterogeneous echogenicity. Therefore, ultrasound appears to have limited predictive value for placental involvement in CMV.

The association between FGR and CMV has been previously reported.[31] In our series, symptomatic newborns had smaller placentas and higher FGR rates than asymptomatic newborns. Stillbirths showed even higher FGR rates, although the difference was not statistically significant, likely due to sample size. FGR may suggest more severe placental and fetal involvement. Pereira et al[31] found significantly more avascular villi, edema, and inflammation in CMV-infected placentas with FGR. Villitis is the primary histopathological feature of CMV infection and is commonly associated with FGR, small or normal-sized placentas, and a low fetal/placental weight ratio.[20] Neonatal weight was lower in symptomatic newborns, although not significantly. PROM occurred in 3 asymptomatic cases, and CMV infection is not typically associated with PROM.[32] Most cases were not premature births. In our center, we did not induce delivery for symptomatic cases unless necessary, to avoid prematurity-related complications. CMV has been associated with preterm birth, although antiviral treatment may reduce this risk.[10,31] All symptomatic cases were singletons. One excluded case involved twins, 1 symptomatic and 1 asymptomatic, but no placenta was available for analysis. This underscores the importance of standardized placental examination.[33] No significant differences in Apgar scores or umbilical cord pH were found, possibly due to the small sample size. Valaciclovir was administered to all newborns, either for 6 weeks or 6 months, based on clinical severity, in accordance with our institutional protocol. This treatment has been shown to reduce both neonatal and 6-month symptoms.[34] More than half of the symptomatic newborns in our study did not exhibit symptoms after 6 months.

The placentas of symptomatic newborns were smaller in size and weight, consistent with previous studies,[8,31] likely reflecting greater CMV-induced dysfunction. Placentomegaly has been described as an early manifestation of inflammatory response, possibly followed by vascular and villous damage. La Torre et al[35] found placental enlargement to be more common in primary infections, with some improvement after hyperimmune globulin. We did not observe an increased frequency of infarcts in any group; when present, they were small, peripheral, and unlikely to significantly affect function. Infarcts are typically associated with maternal malperfusion, found in only 4 asymptomatic cases. Uenaka et al[8] reported similar findings. Gross abnormalities of the umbilical cord or membranes were rare (<30%). CMV infection is associated with acute chorioamnionitis and funisitis.[36] In our series, chorioamnionitis was observed in nearly 50% and funisitis in about 20% of cases. All funisitis cases occurred in the asymptomatic group and were stage 1, grade 1. No statistically significant differences were found, and Uenaka et al[8] also reported lower acute inflammatory lesion rates.

Placental findings vary with gestational age. Chronic villitis is the most frequent histological lesion in nonpremature CMV-infected placentas.[37,38] Chronic lymphoplasmacytic villitis was the most common immune response in our series, significantly more frequent in symptomatic than in asymptomatic cases. When associated with avascular villi, necrosis, breakdown, and hemosiderin deposits, it was significantly more frequent in stillbirth placentas. This may indicate a higher viral load or stronger maternal immune response, leading to widespread villous and vascular injury and ultimately, stillbirth. No differences were found when stratified by grade, possibly due to sample size. Chronic villitis impairs maternal-fetal nutrient exchange and has been linked to adverse neonatal outcomes.[1,39] Chronic plasma cell deciduitis, usually associated with chronic villitis, was also significantly more frequent in symptomatic cases. Uenaka et al[8] reported the opposite finding. Intervillositis was rare, observed in only 1 case, likely in association with high-grade villitis.

Among 16,016 placentas analyzed in our department, 329 were diagnosed with chronic villitis unrelated to CMV. Of these, 29 (8.8%) were attributed to other infections (SARS-CoV-2, toxoplasmosis, Zika, and parvovirus B19); the remainder were classified as villitis of unknown etiology. Only 3 (0.9%) were stillbirths. In our center, CMV PCR is routinely performed on urine in FGR cases, and CMV immunostaining is conducted on all histological villitis cases with negative or unperformed PCR. Potentially missed cases include FGR-negative pregnancies without CMV testing and “normal” placentas or FGR cases with false-negative urine PCR and no pathological placental findings.

Maternal and fetal malperfusion patterns were not observed in symptomatic or asymptomatic newborns because these are not typical of viral placental infections.[1] However, they were present in half of the stillbirths, suggesting a role for villitis-related vascular injury. Fetal thrombotic vasculopathy (fetal vascular malperfusion) has been linked to CMV-related stillbirths,[40] and we observed thrombi in large vessels in all stillbirth placentas. No differences were found in chorangiosis, fibrin deposits, nucleated red blood cells, or dystrophic calcifications between symptomatic and asymptomatic cases. However, stillbirth placentas showed more fibrin deposits (associated with trophoblast damage[41]), nucleated red blood cells (suggesting fetal hypoxia), and membrane changes (possibly indicating higher amniotic viral load). Although dystrophic calcifications were common in symptomatic placentas, they were not detected on ultrasound. These findings are not CMV specific and may occur in other causes of fetal demise, although their distribution and patterns often differ. They are usually present in a diffuse pattern, which was not observed in our cases. Only 3 of all stillbirths in our centers had villitis unrelated to CMV.

CMV inclusions and positive immunostaining were significantly associated with symptomatic newborns and stillbirths. Endothelial and trophoblastic inclusions were more frequent in stillbirths, possibly linked to increased vascular injury. Our

sampling included 3 slides per placenta, and the presence of CMV in these may reflect broader infection and higher fetal risk. CMV detection in the placenta decreases with gestational age,[37] and only approximately 10% of congenital CMV cases show "owl's eye" inclusions,[42] as observed in our series.

Key strengths include a sizable cohort and expert double review of histopathological data. Limitations include the retrospective nature, increased recent referral of placentas due to improved awareness, and missing data from nonsubmitted placentas. Additionally, we did not compare CMV-related findings with other stillbirth causes, and umbilical cord length was not always available.

In conclusion, gross and histological placental abnormalities are associated with symptomatic congenital CMV and stillbirths. More extensive villous and vascular damage appears to facilitate fetal transmission. Treatments targeting placental injury could help prevent CMV-related neonatal symptoms and mortality.

Author Contributions

E.M.P.B, R.M.R.-Z., A.C.-F., and M.D.L.C. performed conception and design of the study and acquisition and analysis of data. E.M.P.B. performed statistical design and analysis. E.M.P.B. and R.M.R.Z. clicked pathology images. C.M.P. clicked radiology images. E.M.P.B., A.C.F., D.M., M.C.B.V., and M.D.L.C. and R.M.R.Z. drafted the manuscript. All authors read and approved the final paper.

Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or nonprofit sectors.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Ethics Approval and Consent to Participate

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Supplementary Material

The online version contains supplementary material available at https://doi.org/10.1016/j.modpat.2025.100808.

References

1. Leruez-Ville M, Chatzakis C, Lilleri D, et al. Consensus recommendation for prenatal, neonatal and postnatal management of congenital cytomegalovirus infection from the European congenital infection initiative (ECCI). *Lancet Reg Health Eur*. 2024;40:100892. https://doi.org/10.1016/j.lanepe.2024.100892
2. Fowler KB, Boppana SB. Congenital cytomegalovirus infection. *Semin Perinatol*. 2018;42(3):149–154. https://doi.org/10.1053/j.semperi.2018.02.002
3. Leruez-Ville M, Foulon I, Pass R, Ville Y. Cytomegalovirus infection during pregnancy: state of the science. *Am J Obstet Gynecol*. 2020;223(3):330–349. https://doi.org/10.1016/j.ajog.2020.02.018
4. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Rev Med Virol*. 2007;17(5):355–363. https://doi.org/10.1002/rmv.544
5. Plotogea M, Isam AJ, Francu F, et al. An overview of cytomegalovirus infection in pregnancy. *Diagnostics (Basel, Switzerland)*. 2022;12(10):2429. https://doi.org/10.3390/diagnostics12102429
6. Pereira L, Maidji E, McDonagh S, Tabata T. Insights into viral transmission at the uterine-placental interface. *Trends Microbiol*. 2005;13(4):164–174. https://doi.org/10.1016/j.tim.2005.02.009
7. Jonjic S. CMV immunology. *Cell Mol Immunol*. 2015;12(2):125–127. https://doi.org/10.1038/cmi.2014.132
8. Uenaka M, Morizane M, Tanimura K, et al. Histopathological analysis of placentas with congenital cytomegalovirus infection. *Placenta*. 2019;75:62–67. https://doi.org/10.1016/j.placenta.2019.01.003
9. Maidji E, Nigro G, Tabata T, et al. Antibody treatment promotes compensation for human cytomegalovirus-induced pathogenesis and a hypoxia-like condition in placentas with congenital infection. *Am J Pathol*. 2010;177(3):1298–1310. https://doi.org/10.2353/ajpath.2010.091210
10. Buxmann H, Stackelberg OM v, Schlößer RL, et al. Use of cytomegalovirus hyperimmunoglobulin for prevention of congenital cytomegalovirus disease: a retrospective analysis. *J Perinat Med*. 2012;40(4):439–446. https://doi.org/10.1515/jpm-2011-0257
11. Bailão LA, Osborne NG, Rizzi MCS, Bonilla-Musoles F, Duarte G, Bailão TCRS. Ultrasound markers of fetal infection part 1: viral infections. *Ultrasound Q*. 2005;21(4):295–308. https://doi.org/10.1097/01.ruq.0000187025.61943.ff
12. Degani S. Sonographic findings in fetal viral infections: a systematic review. *Obstet Gynecol Surv*. 2006;61(5):329–336. https://doi.org/10.1097/01.ogx.0000216518.85796.88
13. Saldan A, Forner G, Mengoli C, Gussetti N, Palù G, Abate D. Testing for cytomegalovirus in pregnancy. *J Clin Microbiol*. 2017;55(3):693–702. https://doi.org/10.1128/JCM.01868-16
14. Diogo MC, Glatter S, Binder J, Kiss H, Prayer D. The MRI spectrum of congenital cytomegalovirus infection. *Prenat Diagn*. 2020;40(1):110–124. https://doi.org/10.1002/pd.5591
15. Pereira L, Tabata T, Petitt M, Fang-Hoover J. Congenital cytomegalovirus infection undermines early development and functions of the human placenta. *Placenta*. 2017;59(Suppl 1):S8–S16. https://doi.org/10.1016/j.placenta.2017.04.020
16. Njue A, Coyne C, Margulis AV, et al. The role of congenital cytomegalovirus infection in adverse birth outcomes: a review of the potential mechanisms. *Viruses*. 2020;13(1):20. https://doi.org/10.3390/v13010020
17. Pesch MH, Mowers J, Huynh A, Schleiss MR. Intrauterine fetal demise, spontaneous abortion and congenital cytomegalovirus: a systematic review of the incidence and histopathologic features. *Viruses*. 2024;16(10):1552. https://doi.org/10.3390/v16101552
18. Crino JP, Driggers RW. Ultrasound findings associated with antepartum viral infection. *Clin Obstet Gynecol*. 2018;61(1):106–121. https://doi.org/10.1097/GRF.0000000000000342
19. Khong TY, Mooney EE, Ariel I, et al. Sampling and definitions of placental lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch Pathol Lab Med*. 2016;140(7):698–713. https://doi.org/10.5858/arpa.2015-0225-CC
20. Redline RW, Ravishankar S, Bagby CM, Saab ST, Zarei S. Four major patterns of placental injury: a stepwise guide for understanding and implementing the 2016 Amsterdam consensus. *Mod Pathol*. 2021;34(6):1074–1092. https://doi.org/10.1038/s41379-021-00747-4
21. Sparić R, Stojković M, Plešinac J, Pecorella G, Malvasi A, Tinelli A. Advanced maternal age (AMA) and pregnancy: a feasible but problematic event. *Arch Gynecol Obstet*. 2024;310(3):1365–1376. https://doi.org/10.1007/s00404-024-07678-w
22. Panda SK, Sahoo M, Pradhan DD. Central hypothyroidism with cytomegalovirus infection in an extremely preterm infant. *Indian J Pediatr*. 2023;90(12):1268, 1268 https://doi.org/10.1007/s12098-023-04783-7
23. Tuli G. Cytomegalovirus infection and congenital hypothyroidism: possible association. *Acta Endocrinol (Buchar)*. 2022;18(1):93–96. https://doi.org/10.4183/aeb.2022.93
24. Schwebke K, Henry K, Balfour HH, Olson D, Crane RT, Jordan MC. Congenital cytomegalovirus infection as a result of nonprimary cytomegalovirus disease in a mother with acquired immunodeficiency syndrome. *J Pediatr*. 1995;126(2):293–295. https://doi.org/10.1016/s0022-3476(95)70563-5
25. Puhakka L, Renko M, Helminen M, et al. Primary versus non-primary maternal cytomegalovirus infection as a cause of symptomatic congenital infection - register-based study from Finland. *Infect Dis (Lond)*. 2017;49(6):445–453. https://doi.org/10.1080/23744235.2017.1279344
26. Rahav G, Gabbay R, Ornoy A, Shechtman S, Arnon J, Diav-Citrin O. Primary versus nonprimary cytomegalovirus infection during pregnancy, Israel. *Emerg Infect Dis*. 2007;13(11):1791–1793. https://doi.org/10.3201/eid1311.061289

27. Revello MG, Lazzarotto T, Guerra B, et al. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. *N Engl J Med*. 2014;370(14):1316–1326. https://doi.org/10.1056/NEJMoa1310214
28. Kagan KO, Enders M, Schampera MS, et al. Prevention of maternal-fetal transmission of cytomegalovirus after primary maternal infection in the first trimester by biweekly hyperimmunoglobulin administration. *Ultrasound Obstet Gynecol*. 2019;53(3):383–389. https://doi.org/10.1002/uog.19164
29. Khalil A, Sotiriadis A, Chaoui R, et al. ISUOG practice guidelines: role of ultrasound in congenital infection. *Ultrasound Obstet Gynecol*. 2020;56(1):128–151. https://doi.org/10.1002/uog.21991
30. Buca D, Di Mascio D, Rizzo G, et al. Outcome of fetuses with congenital cytomegalovirus infection and normal ultrasound at diagnosis: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2021;57(4):551–559. https://doi.org/10.1002/uog.23143
31. Pereira L, Petitt M, Fong A, et al. Intrauterine growth restriction caused by underlying congenital cytomegalovirus infection. *J Infect Dis*. 2014;209(10):1573–1584. https://doi.org/10.1093/infdis/jiu019
32. Bopegamage S, Kacerovsky M, Tambor V, et al. Preterm prelabor rupture of membranes (PPROM) is not associated with presence of viral genomes in the amniotic fluid. *J Clin Virol*. 2013;58(3):559–563. https://doi.org/10.1016/j.jcv.2013.09.013
33. Redline RW, Roberts DJ, Parast MM, et al. Placental pathology is necessary to understand common pregnancy complications and achieve an improved taxonomy of obstetrical disease. *Am J Obstet Gynecol*. 2023;228(2):187–202. https://doi.org/10.1016/j.ajog.2022.08.010
34. Kimberlin DW, Jester PM, Sánchez PJ, et al. Valganciclovir for symptomatic congenital cytomegalovirus disease. *N Engl J Med*. 2015;372(10):933–943. https://doi.org/10.1056/NEJMoa1404599
35. La Torre R, Nigro G, Mazzocco M, Best AM, Adler SP. Placental enlargement in women with primary maternal cytomegalovirus infection is associated with fetal and neonatal disease. *Clin Infect Dis*. 2006;43(8):994–1000. https://doi.org/10.1086/507634
36. Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH, Kim YM. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. *Am J Obstet Gynecol*. 2015;213(4 Suppl):S29–S52. https://doi.org/10.1016/j.ajog.2015.08.040
37. Garcia AG, Fonseca EF, Marques RL, Lobato YY. Placental morphology in cytomegalovirus infection. *Placenta*. 1989;10(1):1–18. https://doi.org/10.1016/0143-4004(89)90002-7
38. Lindholm K, O'Keefe M. Placental cytomegalovirus infection. *Arch Pathol Lab Med*. 2019;143(5):639–642. https://doi.org/10.5858/arpa.2017-0421-RS
39. Sebastian T, Ravikumar G, Crasta J. Villitis of unknown etiology (VUE): effect on placental size and association with clinical parameters. *J Matern Fetal Neonatal Med*. 2022;35(9):1695–1702. https://doi.org/10.1080/14767058.2020.1767577
40. Iwasenko JM, Howard J, Arbuckle S, et al. Human cytomegalovirus infection is detected frequently in stillbirths and is associated with fetal thrombotic vasculopathy. *J Infect Dis*. 2011;203(11):1526–1533. https://doi.org/10.1093/infdis/jir121
41. Taweevisit M, Tansatit M, Sutthiruangwong P, Siranart N, Thorner PS. Combined placental maternal floor infarction and cytomegalovirus placentitis: a case report. *Fetal Pediatr Pathol*. 2022;41(4):651–656. https://doi.org/10.1080/15513815.2020.1857487
42. Sachdev R, Nuovo GJ, Kaplan C, Greco MA. In situ hybridization analysis for cytomegalovirus in chronic villitis. *Pediatr Pathol*. 1990;10(6):909–917. https://doi.org/10.3109/15513819009064726