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Disorders of placental villous maturation in fetal death

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Abstract

Objective: The aims of this study were to ascertain the frequency of disorders of villous maturation in fetal death and to also delineate other placental histopathologic lesions in fetal death.

Methods: This was a retrospective observational cohort study of fetal deaths occurring among women between January 2004 and January 2016 at Hutzel Women's Hospital, Detroit, MI, USA. Cases comprised fetuses with death beyond 20 weeks' gestation. Fetal deaths with congenital anomalies and multiple gestations were excluded.

Controls included pregnant women without medical/obstetrical complications and delivered singleton, term (37–42 weeks) neonate with 5-min Apgar score ≥ 7 and birthweight between the 10th and 90th percentiles.

Results: Ninety-two percent (132/143) of placentas with fetal death showed placental histologic lesions. Fetal deaths were associated with (1) higher frequency of disorders of villous maturation [44.0% (64/143) vs. 1.0% (4/405), $P < 0.0001$, prevalence ratio, 44.6; delayed villous maturation, 22% (31/143); accelerated villous maturation, 20% (28/143); and maturation arrest, 4% (5/143)]; (2) higher frequency of maternal vascular malperfusion lesions [75.5% (108/143) vs. 35.7% (337/944), $P < 0.0001$, prevalence ratio, 2.1] and fetal vascular malperfusion lesions [88.1% (126/143) vs. 19.7% (186/944), $P < 0.0001$,

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prevalence ratio, 4.5]; (3) higher frequency of placental histologic patterns suggestive of hypoxia [59.0% (85/143) vs. 9.3% (82/942), $P < 0.0001$, prevalence ratio, 6.8]; and (4) higher frequency of chronic inflammatory lesions [53.1% (76/143) vs. 29.9% (282/944), $P < 0.001$, prevalence ratio 1.8].

Conclusion: This study demonstrates that placentas of women with fetal death were 44 times more likely to present disorders of villous maturation compared to placentas of those with normal pregnancy. This suggests that the burden of placental disorders of villous maturation lesions is substantial.

Keywords: accelerated villous maturation; CD15; delayed villous maturation; fetal hypoxia; fetal vascular malperfusion; hypercapillarized villi; intravillous hemorrhage; maternal vascular malperfusion; nucleated red blood cells; placental inflammatory lesions.

Introduction

Approximately 2.6 million fetal deaths >28 weeks of gestation occurred worldwide in 2015 [1]. The rate of fetal death in the United States is 5.96 per 1000 live births [2], and it is twice more common in African-American than in Caucasian women [2, 3]. Complications of pregnancy associated with fetal death include maternal hypertension [4–10], diabetes mellitus [4, 11, 12], intrauterine infection [13–15] or inflammation [16–19], and placental abruption [4, 8, 20–25].

The American College of Obstetricians and Gynecologists Committee on Genetics has recommended that placental examination should become a routine clinical tool in stillbirth evaluation [26, 27]. The committee recommended gross and microscopic examination of the

placenta, including the membranes and umbilical cord, to gain information about the potential causes of fetal death [26]. Placental pathology in fetal death is now deemed as a research priority to gain insights into the causes and mechanisms of fetal death [27–29].

Surgical examination of the placenta has been largely aimed to identify the traditional mechanisms of disease that affect any organ, such as acute or chronic inflammation, vascular pathology, in rare occasions, tumors, or other abnormalities. However, the placenta is the subject of profound developmental changes during its lifespan. The villous tree undergoes changes in morphology and components, which are collectively referred as villous maturation. This process is characterized by a steadily increasing number of villous capillaries and formation of syncytiocapillary membranes within terminal chorionic villi [30]. The apposition of syncytiotrophoblasts with villous capillary endothelium and formation of vasculosyncytial membranes shortens the distance between the fetal and maternal blood supplies. This is the main mechanism by which the placenta meets growing fetal demand in the last 2 months of pregnancy [30]. Thus, defective villous tree maturation can cause diminution of vasculosyncytial membranes resulting in placental dysfunction and fetal hypoxia [31–36]. Disorders of villous maturation are ramification disorders of the villous tree whereby the observed morphology is chronologically deviated from what should be normal for the gestational age [36–39] and are associated with considerable morbidity and even mortality [31, 36, 40–43]. Vogel has proposed that villous maturation disorders affecting 50% or more villi are pathophysiologically relevant [36, 39].

Given that there are no studies that have evaluated disorders of villous maturation in placentas of structurally normal stillbirths, the purpose of this study was to

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utilize CD15 immunohistochemistry to objectively diagnose delayed villous maturation. We also performed a systematic evaluation of the placentas of fetal death using standardized placental nomenclature [31, 44–53], according to the recommendations of the Amsterdam Placental Workshop Group, in order to understand the context in which placental maturational disorders fit with other placenta pathologic lesions.

Materials and methods

This was a retrospective observational study of fetal deaths that occurred among pregnant women recruited into cohort studies conducted between January 2004 and January 2016 at Hutzel Women's Hospital of the Detroit Medical Center Detroit, MI, USA. All study participants provided written informed consent prior to sample collection and were followed until delivery. The use of clinical data and biological specimens obtained from these women for research purposes was approved by the Human Investigation Committee of Wayne State University.

Cases consisted of placentas from fetuses who died between 20 and 41 weeks of gestation (derived from last menstrual period and/or fetal biometry) [54]. There were 206 fetal deaths during this study period. Of these, 38 with congenital anomalies and 12 twin pregnancies were excluded from the study. An additional 13 cases were excluded as they did not have sufficient clinical information available. There were 143 fetal deaths and 944 controls. Controls included placentas from pregnant women who had no medical or obstetrical complications and delivered a singleton, term (37–42 weeks) neonate with a 5-min Apgar score ≥ 7 and birthweight between the 10th and 90th percentiles, according to a U.S. reference population [55].

Excluded from the control group were pregnant women who presented (1) maternal chronic diseases (e.g. chronic hypertension, diabetes mellitus, renal disease, thyroid disease, asthma, autoimmune disease, and coagulopathies) and (2) pregnancy complications (e.g. gestational hypertension, preeclampsia, eclampsia, placental abruption, preterm labor, preterm prelabor rupture of the fetal membranes, gestational diabetes mellitus, clinical chorioamnionitis, intrapartum fever, fetal malformations, chromosomal anomalies, multifetal gestation, fetal death, and HELLP syndrome [abbreviated for its indicators: hemolysis, elevated liver enzymes, and low platelet count]).

Placental histopathological study

The placenta was sampled using systematic random and targeting sampling techniques as previously described [56]. Slides from the cases and controls, stained with hematoxylin and eosin, as well as cytokeratin-7, were reviewed by a placental pathologist blinded to the pregnancy outcome and any previous histopathological diagnoses. The nomenclature recommended by the Amsterdam Placental Workshop Group was used to describe the placental lesions [57], except for maturation arrest [36–39] and placental injury demonstrating histologic patterns suggestive of hypoxia.

Slides were also stained for CD15 immunohistochemistry whereby the “immature” CD15-positive endothelium was considered as a diagnostic marker of persisting villous immaturity and chronic placental dysfunction [58]. The level of CD15 expression in both the macrovasculature (chorionic plate and stem vessels) and microvasculature (terminal villi) with $\geq 50\%$ villi exhibiting positive expression was considered as pathologic placental villous immaturity [58]. Two blocks each from fetal death cases ($n = 143$) as well as placentas from normal pregnancy outcome (controls, $n = 405$) were stained with CD15. The technique described by Seidmann [58, 59] was followed whereby blood vessels of the chorionic villi, stem villi, and chorionic plate were counted and the ratio was calculated as positively stained endothelial cells over total number of vessels counted [58, 59]. Only those blood vessels that showed circumferential staining reaction (all the endothelial cells in a vessel) were considered positive. A section was first scanned using a $4\times$ objective, and only those cases where 50% of the chorionic villi in a section showed positive staining reaction, further 500 blood vessels were counted.

Immunohistochemistry was also used to confirm both cytomegalovirus (CMV) and parvovirus infection and was performed by the streptavidin-biotin complex method using a monoclonal antibody (Leica-Bond, Buffalo Grove, IL, USA).

Placental histopathological definitions are displayed in Table 1.

A schematic representation of normal villous tree and disorders of villous maturation depicted in Figure 1.

Statistical analysis

The Welch's *t*-test was used to compare the means of continuous variables between groups (Fetal death vs. normal), and for categorical variables, proportions were compared using Fisher's exact test. The prevalence ratio was calculated for each placental lesion as the ratio of the frequency of lesions in fetal deaths to that in the normal controls. The Benjamini-Hochberg procedure [102] was employed to adjust for multiple comparisons. A *q*-value (adjusted *P*-value) of less than 0.05 was considered statistically significant. Statistical analyses were performed using R [103].

Results

Demographic and clinical characteristics of the study population

There were 944 controls and 143 fetal deaths. The demographic and clinical characteristics of the study population are shown in Table 2. Mean maternal age, pre-pregnancy body mass index, systolic and diastolic blood pressure, and mean arterial blood pressure were higher in women with fetal death than those with a normal pregnancy outcome. Moreover, women with fetal death showed significantly higher African-American ethnicity, maternal obesity, tobacco use, alcohol use, drug abuse, history of

Table 1: Pathology definitions.

Accelerated villous maturation	Defined as the presence of small or short hypermature villi for gestational period and usually accompanied by an increase in syncytial knots and considered to reflect maternal vascular malperfusion [35, 60, 61]. With earlier and more severe type of maternal vascular malperfusion, premature placentas show distal villous hypoplasia, characterized by missing branching of mature intermediate and terminal villi [35]. The histologic indicator for accelerated villous maturation is an alternating pattern of villous crowding and paucity in a low power examination [62]
Delayed villous maturation	Defined by the presence of a monotonous villous population (at least 10 such villi) with centrally placed capillaries and decreased vasculosyncytial membranes, recapitulating the histology of early pregnancy and involving 30% of one full-thickness parenchymal slide [35]
Maturation arrest	Defined as the presence of primitive mesenchymal, embryonic, and loose reticular stroma with Hofbauer cells [36–38]. Capillaries are few, with only rare contact to the surface epithelium and thereby considerably reduced vasculosyncytial membranes and the stem villi are narrow with reduced vascular smooth muscle cells [36–39]
Placental histologic patterns suggestive of hypoxia	Lesions demonstrating histologic patterns suggestive of hypoxia included nucleated red blood cells, hypercapillarized villi, intravillous hemorrhage, massive perivillous fibrinoid deposition, and laminar necrosis of the decidua capsularis. Nuclear red blood cells, hypercapillarized villi, and intravillous hemorrhage represent compensatory change due to fetal hypoxia, while massive perivillous fibrinoid deposition and laminar necrosis represent cellular hypoxia
Nucleated red blood cells	Defined by the presence of increased circulating nucleated red blood cells identified by the presence of hyperchromatic, round nuclei with dense chromatin and polychromatic pink-gray cytoplasm [63]
Hypercapillarization or hypervascularity of villi	Characterized by the presence of an increased number of vascular channels, greater than five but less than ten, in terminal villi, which were differentiated from congestion or dilation [64]
Intravillous hemorrhage	Defined by the presence of extravasated red blood cells in the chorionic villous stroma [65, 66]
Laminar necrosis of the placental membranes	Defined by the presence of a band of coagulative necrosis at the choriodecidual interface of the placental membranes, involving at least 10% of the membrane roll [64, 67–69]
Massive perivillous fibrinoid deposition	Defined by the presence of extensive deposition of fibrinoid material in the intervillous space encasing and strangulating the chorionic villi [70]. Three patterns of massive perivillous fibrinoid deposition have been described: classic, borderline, and transmural [70]. Classic – encasement of basal villi by fibrinoid along the entire maternal floor and of ≥ 3 mm thickness on at least one slide. Borderline massive perivillous fibrinoid deposition-involvement of 25–50% of villi on at least one slide with transmural or nearly transmural distribution. Transmural massive perivillous fibrinoid deposition-transmural perivillous fibrinoid extension, with encasement of $\geq 50\%$ of villi on at least one slide
Cytomegalovirus (CMV) placentitis	Defined by the presence of focal segmental chronic lymphoplasmacytic villitis, hemosiderin deposition, and an occasional presence of owl-like inclusions in the stromal cells, determined by light microscopy with hematoxylin and eosin staining [71, 72]. The diagnosis of CMV placentitis was subsequently confirmed by immunohistochemistry [73–75]. Cases of chronic villitis without lymphoplasmacytes in the villi, showing no evidence of owl-like inclusions and lacking hemosiderin deposition and which were negative for immunohistochemistry were not considered as CMV villitis
Parvovirus placentitis	Defined by the presence of intra-nuclear brick-red inclusions in erythroblasts [76, 77]. The inclusions found in the umbilical cord and the fetal vessels of the chorionic plate or stem villi, or within capillaries of the terminal villi, were considered indicative of parvovirus placentitis [78–83]. The diagnosis was confirmed by immunohistochemistry
Fetal maceration	Defined as a process that occurs following fetal death and is characterized by enzymatic autolysis of cells and degeneration of connective tissue leading to skin discoloration, desquamation with formation of bullae and eventually skin peeling, as well as edema of the outer and inner organs with turbid effusions inside the fetal body and amniotic cavity [84]

Table 1 (continued)

Maternal vascular malperfusion	<p>Maternal vascular malperfusion diagnosis represents a constellation of findings involving the villous parenchyma and/or involving maternal decidual vasculature [57, 85–90]</p> <p>Villous changes</p> <p>Villous infarct(s): dead villous tissue in which the trophoblastic nuclei are clustered and necrotic with clumped partly dispersed DNA (recent), or the intervillous space is collapsed, the villi are crowded together, and there is complete disappearance of nuclear DNA</p> <p>Increased syncytial knots: aggregates of syncytiotrophoblasts nuclei along proximal stem villi or at one or more poles of distal villi</p> <p>Villous agglutination: clusters of adherent distal villi agglutinated by fibrin and/or bridging syncytial knots</p> <p>Increased intervillous fibrin: abnormal amounts of intervillous fibrinoid either coat proximal stem villi or are eccentrically adherent to (or within, following re-epithelialization) distal villi</p> <p>Distal villous hypoplasia: paucity of villi in relation to the surrounding stem villi. The villi are thin and relatively elongated-appearing, and syncytial knots are increased. The features are seen in lower two-thirds and involve at least 30% of one full-thickness parenchymal slide</p> <p>Vascular lesions</p> <p>Persistent muscularization of basal plate arteries: persistence of smooth muscle cells in the wall of a large spiral artery in the basal plate</p> <p>Mural hypertrophy of decidual arterioles: thickening of the muscular wall of arterioles in the extraplacental membranes</p> <p>Acute atherosclerosis of basal plate arteries and/or decidual arterioles: eosinophilic fibrinoid necrosis of arterial smooth muscle and presence of subendothelial or medial foam cells (macrophages) in maternal arteries</p> <p>Fetal vascular malperfusion is a term applied to a group of placental lesions indicating reduced or absent perfusion of the villous parenchyma by the fetus [35, 36, 57, 62, 90–95]</p> <p>Villous changes</p> <p>Early: villous stromal-vascular karyorrhexis (three or more foci of two or more terminal villi showing karyorrhexis of fetal cells with preservation of surrounding trophoblast)</p> <p>Late: hyalinized avascular villi, either as exclusively small foci or variable-sized foci</p> <p>Severe (high grade): consistent with fetal thrombotic vasculopathy with an average of 15 or more affected villi per slide</p> <p>Vascular lesions</p> <p>Thrombi large fetal vessels: organized blood clot, occlusive or non-occlusive, of any age (defined by two or more of the following: fibrin strands, glassy texture with slight hematoxylin blush, adherence to endothelium) compromising the lumen of fetal vessels in the chorionic plate or proximal origin of the villous tree</p> <p>Intimal fibrin deposition, large fetal vessels: fibrin or fibrinoid deposition (subendothelial or intramuscular) within the wall of large fetal vessels</p> <p>Fibromuscular sclerosis, intermediate-sized fetal vessels: abnormal fetal stem vessels caused by loss of fetal circulation, resulting in thickening of the muscular wall of vessels with progressive obliteration of lumen and a loss of endothelial lining</p> <p>Chronic inflammatory lesions of the placenta are characterized by the infiltration by lymphocytes, plasma cells and/or macrophages, which may be a result of infections (viral, bacterial, parasitic) or may be of immune origin (maternal anti-fetal rejection). Chronic villitis (when the inflammatory process affects the villous tree), chronic chorioamnionitis (which affects the chorioamniotic membranes), and chronic deciduitis (which involves the decidua basalis) are the three types described [35, 57, 62, 90, 96, 97]</p> <p>Villitis of unknown etiology: defined as a destructive chronic inflammatory lesion of the chorionic villi characterized by an infiltrate of maternal T lymphocytes in the villous stroma and an elevated numbers of fetal macrophages (Hofbauer cells) [98, 99]</p> <p>Low grade: presence of chronic inflammation affecting fewer than ten contiguous villi in any one focus; focal (one slide, fewer than 10 villi) or multifocal (more than one slide)</p> <p>High grade: presence of multiple foci, on more than one section, at least one of which shows inflammation affecting more than ten contiguous villi; patchy (multiple foci, with at least one focus with ten or more contiguous villi) or diffuse (more than 30% of all distal villi are involved)</p> <p>Basal villitis: predominantly involving anchoring villi and adjacent terminal villi</p> <p>Proximal villitis: involving distal villi or proximal stem villi</p> <p>Chronic deciduitis with plasma cells: a diffuse lymphocytic infiltration of the decidua or any infiltrate accompanied by plasma cells</p>
Fetal vascular malperfusion	
Chronic placental inflammation	

Table 1 (continued)

Acute chorioamnionitis and funisitis	Acute inflammatory lesions of the placenta are defined by diffuse infiltration of neutrophils and include acute chorioamnionitis, funisitis, and chorionic vasculitis representing a host response (maternal or fetal) to a chemotactic gradient in the amniotic cavity [35, 36, 57, 62, 90, 100, 101]
	Maternal inflammatory response
	Stage 1: acute subchorionitis/acute chorionitis with accumulations of neutrophils in the subchorionic zone and/or in the chorionic trophoblast layer of the extraplacental membranes
	Stage 2: acute chorioamnionitis with more than a few scattered neutrophils in the chorionic plate and membranous connective tissues and/or in the amnion
	Stage 3: necrotizing or subacute chorioamnionitis with a marked neutrophilic infiltrate with degenerating neutrophils (karyorrhexis), thickened eosinophilic amniotic basement membrane, and focal amniotic epithelial necrosis
	Grade 1: not severe as defined
	Grade 2: severe: confluent polymorphonuclear leukocytes or with subchorionic microabscesses
	Fetal inflammatory response
	Stage 1: umbilical phlebitis/chorionic vasculitis with neutrophils identified in the wall of any chorionic plate vessel or in the umbilical vein
	Stage 2: umbilical arteritis with neutrophils seen in one or both umbilical arteries and/or the umbilical vein
	Stage 3: necrotizing funisitis with neutrophils, cellular debris, and/or mineralization present in a concentric band, ring, or halo around one or more umbilical vessels
	Grade 1: not severe as defined
	Grade 2: severe: near-confluent intramural polymorphonuclear leukocytes with attenuation of vascular smooth muscle

preterm birth, induction of labor, and birth-weight <75th percentile as compared to controls.

Cesarean delivery, spontaneous labor, gestational age at delivery, and fetal birth-weight were significantly lower in women with fetal death as compared to controls (Table 2).

Risk factors for fetal death

More than one risk factor was noted in 80 cases (56.0%), 47 cases (32.9%) had only one risk factor, and 16 cases (11.2%) did not have any risk factors. Pre-existing maternal complications were observed in 72 cases (50.3%), of which 24 cases harbored more than one morbid factor, which included maternal medical conditions. Pregnancy-related risk factors included 107 cases (74.8%), of which 53 were hypertensive disorders of pregnancy [11 (7.4%) preeclampsia, 2 (1.4%) HELLP syndrome, 14 (9.8%) chronic hypertension, 17 (11.9%) chronic hypertension with preeclampsia, and 10 (7.0%) gestational hypertension]; 37.1% (52/140) were small-for-gestational-age neonates; 12.8% (18/140) had placental abruption, 4.2% (6/140) had pregestational diabetes and 7.0% (10/140) had gestational diabetes, 21.2% (14/66) had intra-amniotic infection, 45.5% (20/44) had intra-amniotic inflammation, and 4.1% (6/143) had villitis of infectious origin, which included 4 cases of parvovirus B19 infection and 2 with CMV infection.

Gross placental findings

The mean value of the following gross placental characteristics was lower in the cases of fetal death than in the controls: the shortest distance from the umbilical cord insertion to the margin of placental disc, the frequency of marginal umbilical cord insertion, and the frequency of circummarginate placental membranes ($P < 0.0001$ for all variables) (Table 3).

Microscopic placental findings

Disorders of villous maturation

Disorders of villous maturation were significantly higher in the fetal death cases as compared to the controls [44.0% (63/143) vs. 1% (4/405), $q < 0.0001$; prevalence ratio, 44.6; delayed villous maturation, 22% (31/143); accelerated villous maturation, 20% (28/143); and maturation arrest,

Figure 1: Schematic representation of normal term villous tree and disorders of villous maturation.

Brown: trunk of a tree depicting stem villi; orange: intermediate trunk of a tree depicting immature intermediate villi; yellow: branches of a tree depicting mature intermediate villi; and green: leaves of a tree depicting terminal villi. At term, the villous tree is constituted by terminal villi, mature intermediate villi, stem villi, and even mesenchymal villi [88] (0–5%; not depicted here). Delayed villous maturation is characterized by medium-sized intermediate villi of peripheral mature type along with stem villi. The villi display reticular stroma, centralized vessels, and diminished vasculo-syncytial membranes. Maturation arrest is characterized by the predominance of immature intermediate villi displaying primitive mesenchymal, embryonic, and loose reticular stroma with Hofbauer cells, few capillaries, considerably reduced vasculosyncytial membranes, and diminished intervillous space. Accelerated villous maturation is characterized by hypermature, hypoplastic, and slender terminal villi resembling the histology of term villi with considerably increased intervillous space.

Table 2: Maternal characteristics and perinatal outcomes in normal pregnancy and fetal death.

	Normal pregnancy (n=944)	Fetal death (n=143)	P-value
Maternal characteristics			
Maternal age, years	24.8 (15–42)	27 (15–44)	0.0001
Nulliparity	320/944 (33.9%)	47/143 (32.9%)	0.84
Maternal height, cm	163.1 (137.2–185.4)	163.4 (119.4–190.5)	0.66
Pre-pregnancy BMI, kg/m ²	27.2 (15.5–67.4)	30.7 (16.6–56.6)	0.0001
Obesity (≥ 30 kg/m ²)	257/886 (29%)	54/121 (44.0%)	<0.001
Ethnicity			<0.05
African American	764/943 (81%)	128/143 (89.5%)	
Caucasian	83/943 (8.8%)	6/143 (4.2%)	
Others	96/943 (10.2%)	9/143 (6.3%)	
Blood pressure at enrollment (mm Hg)			
Systolic	128.5 (82–175)	140.2 (102–229)	<0.0001
Diastolic	75 (40–101)	82.3 (49–146)	<0.0001
Mean arterial blood pressure	92.8 (64–120.7)	101.6 (69.3–173.7)	<0.0001
Tobacco use during pregnancy	111/940 (11.8%)	45/143 (31.5%)	<0.0001
Alcohol use during pregnancy	7/939 (0.7%)	8/143 (5.6%)	<0.001
Drug abuse	118/942 (12.5%)	43/143 (30.1%)	<0.0001
History of preterm births	68/944 (7.2%)	39/143 (27.3%)	<0.0001
Perinatal outcomes			
Gestational age at delivery, weeks	39.6 (37–42)	29.9 (20.3–40.9)	<0.0001
Cesarean delivery	247/944 (26.2%)	10/143 (7.0%)	<0.0001
Vaginal delivery	670/944 (71%)	117/148 (81.8%)	<0.001
Spontaneous labor	408/944 (43.2%)	13/143 (9.1%)	<0.0001
Induction of labor	137/944 (14.5%)	117/143 (81.8%)	<0.005
Birth weight, g	3345.7 (2560–4090)	1427.8 (48–4706)	<0.0001
Birthweight <75 th percentile	848/944 (89.8%)	137/140 (97.9%)	<0.002
Birthweight <50 th percentile	548/944 (58.1%)	117/140 (83.6%)	<0.0001
Birthweight <25 th percentile	154/944 (16.3%)	84/140 (60.0%)	<0.0001
Birthweight <10 th percentile	0/944 (0%)	52/140 (37.1%)	<0.001
Male sex neonate	486/940 (51.7%)	77/136 (56.6%)	0.31

Data are presented as median (range) or n (%). BMI, body mass index.

Table 3: Placentas on gross examination of normal pregnancy and pregnancy with fetal death.

Characteristics	Normal pregnancy (n=944)	Fetal death (n=143)	P-value
Placental weight, untrimmed, g	571.2 (324–989)	335.8 0 (41–1032)	<0.0001
Placental weight <10 th percentile	224/930 (24.1%)	42/108 (38.9%)	<0.001
Fetoplacental weight ratio	6 (3–10.2)	3.9 (0.6–9.9)	<0.0001
Fetoplacental weight ratio <10 th percentile	11/930 (1.2%)	7/106 (6.6%)	0.001
Placental disc dimensions			
Placental disc length, cm	18.3 (12–32)	14.5 (6.5–29.5)	<0.0001
Placental disc width, cm	15.7 (5.2–22)	12.4 (2–19.5)	<0.0001
Placental thickness, cm	2.4 (0.5–4)	2.1 (0.2–4)	<0.0001
Placental volume, cm ³	689.6 (150–1840)	406.0 (19.8–1872)	<0.0001
Shortest distance of the umbilical cord insertion to the margin, cm	5.5 (0.3–10)	4.1 (0.3–15)	<0.0001
Umbilical cord abnormality			
Marginal insertion	11/938 (1.2%)	25/143 (17.5%)	<0.0001

Data are presented as median (range) or n (%).

4% (5/143)] (Table 4; Figure 2). CD15 to ascertain delayed villous maturation was performed on all the fetal death cases and in 405/944 controls.

Acute placental inflammation

The frequency of severe acute chorioamnionitis (a maternal inflammatory response of stage 3 necrotizing chorioamnionitis and/or grade 2 severe subchorionic microabscess) was significantly higher in cases of fetal death than in the controls [6.3% (9/143) vs. 0.7% (7/944), $q < 0.0001$; prevalence ratio, 8.5].

The frequency of acute fetal inflammatory response was significantly lower in the cases of fetal death than in the controls [8.5% (12/142) vs. 23.2% (219/944), $q < 0.0001$; prevalence ratio, 0.4]. Specifically, the frequency of acute chorionic vasculitis or umbilical phlebitis was significantly lower in the cases of fetal death than in the controls [4.2% (6/142) vs. 18.8% (177/944), $q < 0.0001$; prevalence ratio, 0.2] (Table 5). The prevalence of fetal inflammatory response compared to the prevalence of maternal inflammatory response in fetal deaths was 12/142 (8.5%) vs. 52/143 (36.4%); $P < 0.001$.

Chronic placental inflammation

The cases of fetal death had a higher frequency of chronic inflammatory lesions than the controls [53.1% (76/143) vs. 29.9% (282/944), $q < 0.0001$; prevalence ratio, 1.8]; specifically, the frequency of chronic chorioamnionitis grade 1/ stage 1, chronic villitis of infectious origin (diagnosed by immunohistochemistry for CMV and parvovirus), and chronic deciduitis was higher in the cases than the controls (Table 5). On the other hand, the frequency of *low-grade* villitis of unknown etiology was lower in cases of fetal death than the controls [10.5% (15/143) vs. 18.5% (175/944), $q < 0.02$; prevalence ratio, 0.6].

Fetal vascular malperfusion lesions

The frequency of placental lesions of fetal vascular malperfusion (both villous and vascular) was higher in the cases of fetal death than the controls [88.1% (126/143) vs. 19.7% (186/944), $q < 0.0001$; prevalence ratio, 4.5]. Except for the frequency of hyalinized avascular villi of small foci, the frequency of all specific lesions categorized as fetal vascular malperfusion was higher in cases of fetal death compared to the controls (Table 5).

Table 4: Frequency of disorders of villous maturation.

Disorders of villous maturation	Normal pregnancy (n=944)	Pregnancy with fetal death (n=143)	q-Value	PR
Prevalence	4/405 (1.0%)	63/143 (44%)	<0.0001	44.6
Delayed villous maturation	4/405 (1%)	31/143 (22%)	<0.0001	21.9
Maturation arrest	1/942 (0.1%)	5/143 (4%)	<0.0003	32.9
Accelerated villous maturation	0/405 (0%)	28/143 (20%)	<0.0001	

Data are presented as n (%). PR, prevalence ratio.

Figure 2: Histology of normal term placenta compared to disorders of villous maturation.

Panel I – normal term placenta (40 weeks); panel II – delayed villous maturation (38.6 weeks); panel III – maturation arrest (32 weeks); panel IV – accelerated villous maturation (33 weeks). (A), (B) and (C) H&E-stained sections at 40, 100, and 400 $\times$ magnifications, respectively. (D) Immunohistochemistry of CD15-stained sections at 100 $\times$ magnification. I: normal term placenta. (A) Normal chorionic plate (blue star) with subchorionic villi that are mature and appropriate for gestational age; intervillous space is white space (red star). (B) Normal term vascularized villi with intervillous space (white; red star). (C) Mature terminal villi with arrows pointing toward vasculo-syncytial membranes formed by the apposition of syncytiotrophoblasts with villous capillary endothelium. (D) Chorionic villous capillary endothelium negative for CD15 staining (absence of brown staining). II: delayed villous maturation. (A) Chorionic plate (blue star) with sub-chorionic villi that are crowded, immature, affecting >50% of placental villous population and resembling the histology of second trimester villi. Intervillous space is considerably diminished (white space as compared to normal in panel I). (B) Virtually absent intervillous space (red star). (C) The villi display more stroma (blue star), centralized vessels (red arrows), and paucity of vasculo-syncytial membranes (blue arrow) as compared to normal pregnancy at similar gestational age (refer to panel I). (D) CD15-positive (brown staining) capillary endothelium in stem (red star) and mature intermediate chorionic villi. III: maturation arrest. (A) and (B) Predominantly immature intermediate villi displaying primitive mesenchymal, embryonic, and loose reticular stroma with Hofbauer cells with diminished intervillous space (red star). (C) Villi show few capillaries (red arrow) with considerably reduced vasculosyncytial membranes, loose stroma (blue star), and Hofbauer cells (blue arrow). (D) CD15 positive chorionic vessels (red arrow; brown staining). IV: accelerated villous maturation. (A) Chorionic plate (blue star) with sub-chorionic villi displaying histology resembling that of term villi (refer to panel I) with considerably increased intervillous space (white space; red star) for the gestational age. (B) The terminal villi appear slender, hypoplastic (red arrows), and mature intermediate villi (blue arrow) and stem villi (red star) also seen in the section. (C) Villous syncytial knotting (blue arrows) and hypermature hypoplastic slender terminal villi (red arrow). (D) Capillary endothelium of chorionic villi negative for CD15 staining (absence of brown staining).

Placental injury demonstrating histologic patterns suggestive of hypoxia

Lesions demonstrating histologic patterns suggestive of hypoxia included nucleated red blood cells, hypercapillarized villi, intravillous hemorrhage, massive perivillous fibrinoid deposition, and laminar necrosis of the decidua capsularis. The frequency of these lesions was seven times higher in the cases than the normal pregnancy controls

[cases, 59% (85/143) vs. controls, 9% (82/942), $q < 0.0001$] (Table 5).

Fetal death without histologic placental lesions

In the current study, 11 cases of fetal death did not show significant placental pathologic lesions (Table 6).

Table 5: Frequency of placental histologic lesions in normal pregnancy and pregnancy with fetal death.

Histologic lesions	Normal pregnancy (n=944)	Fetal death (n=143)	q-Value	PR
Acute inflammatory lesions	399/944 (42.3%)	55/143 (38.5%)	0.50	0.9 (0.7–1.1)
Maternal inflammatory response	341/944 (36.1%)	52/143 (36.4%)	1.0	1 (0.8–1.3)
Stage 1: early acute subchorionitis or chorionitis	220/944 (23.3%)	24/143 (16.8%)	0.11	0.7 (0.5–1.1)
Stage 2: acute chorioamnionitis	114/944 (12.1%)	23/143 (16.1%)	0.22	1.3 (0.9–2)
Stage 3: necrotizing chorioamnionitis	7/944 (0.7%)	5/143 (3.5%)	0.02	4.7 (1.5–14.7)
Severe: stage 3 and/or grade 2	7/944 (0.7%)	9/143 (6.3%)	<0.0001	8.5 (3.2–22.4)
Fetal inflammatory response	219/944 (23.2%)	12/142 (8.5%)	<0.0001	0.4 (0.2–0.6)
Stage 1: chorionic vasculitis or umbilical phlebitis	177/944 (18.8%)	6/142 (4.2%)	<0.0001	0.2 (0.1–0.5)
Stage 2: umbilical arteritis	41/944 (4.3%)	4/142 (2.8%)	0.58	0.6
Stage 3: necrotizing funisitis	1/944 (0.1%)	2/147 (1.4%)	0.07	13.3
Severe: stage 3 and/or grade 2	2/944 (0.2%)	3/142 (2.1%)	<0.02	10
Chronic inflammatory lesions	282/944 (29.9%)	76/143 (53.1%)	<0.0001	1.8 (1.5–2.1)
Chronic deciduitis	182/944 (19.3%)	56/143 (39.2%)	<0.0001	2 (1.6–2.6)
Lymphocytic (without plasma cells)	97/944 (10.3%)	32/143 (22.4%)	<0.0002	2.2 (1.5–3.1)
Lymphoplasmacytic	85/944 (9%)	24/143 (16.8%)	<0.01	1.9 (1.2–2.8)
Villitis of unknown etiology (VUE)	175/944 (18.5%)	15/143 (10.5%)	<0.02	0.6 (0.3–0.9)
Low-grade lesions	121/925 (13.1%)	12/143 (8.4%)	0.17	0.6 (0.4–1.1)
High-grade lesions	11/925 (1.2%)	3/143 (2.1%)	0.50	1.8
Chronic chorioamnionitis	120/944 (12.7%)	28/143 (19.6%)	0.05	1.5 (1.1–2.2)
Grade 1/stage 1	34/940 (3.6%)	24/143 (16.8%)	<0.0001	4.6 (2.8–7.6)
Grade 1/stage 2	62/940 (6.6%)	4/143 (2.8%)	0.12	0.4
Grade 2/stage 1	2/940 (0.2%)	0/143 (0%)	1.0	0
Grade 2/stage 2	10/940 (1.1%)	0/143 (0%)	0.45	0
Chronic histiocytic intervillitis	4/942 (0.4%)	2/143 (1.4%)	0.23	3.3
Villitis of infectious origin	0/942 (0%)	6/143 (4.2%)	<0.0001	–
Eosinophilic T-cell vasculitis	4/942 (0.4%)	2/143 (1.4%)	0.22	3.3
Severe chronic inflammatory lesions ^a	23/921 (2.5%)	3/143 (2.1%)	1.0	0.8
Maternal vascular malperfusion	337/944 (35.7%)	108/143 (75.5%)	<0.0001	2.1 (1.9–2.4)
Villous changes	176/944 (18.6%)	63/143 (44.1%)	<0.0001	2.4 (1.9–3)
Villous infarct(s)	53/943 (5.6%)	50/143 (35.0%)	<0.0001	6.2 (4.4–8.8)
Increased syncytial knots	80/944 (8.5%)	26/143 (18.2%)	<0.001	2.1 (1.4–3.2)
Villous agglutination	19/944 (2%)	22/143 (15.4%)	<0.0001	7.6 (4.2–13.8)
Increased intervillous fibrin deposition	60/944 (6.4%)	13/143 (9.1%)	0.26	1.4 (0.8–2.5)
Distal villous hypoplasia	1/944 (0.1%)	16/143 (11.2%)	<0.0001	105.6
Vascular lesions	194/944 (20.6%)	92/143 (64.3%)	<0.0001	3.1 (2.6–3.7)
Persistent muscularization of basal plate arteries	154/944 (16.3%)	41/143 (28.7%)	0.001	1.8 (1.3–2.4)
Mural hypertrophy of decidual arterioles	17/944 (1.8%)	31/143 (21.7%)	<0.0001	12 (6.8–21.2)
Acute atherosclerosis of basal plate arteries and/or decidual arterioles	8/944 (0.8%)	19/143 (13.3%)	<0.0001	15.7 (7–35.1)
Spiral artery fibrinoid necrosis	3/942 (0.3%)	27/143 (18.9%)	<0.0001	59.3
Decidual vascular thrombosis	16/942 (1.7%)	24/143 (16.8%)	<0.0001	9.9 (5.4–18.1)
Persistence of endovascular trophoblast	3/942 (0.3%)	21/143 (14.7%)	<0.0001	46.1
Retroplacental hemorrhage	8/942 (0.8%)	30/143 (21.0%)	<0.0001	24.7 (11.6–52.8)
≥ 2 lesions of maternal vascular malperfusion	70/944 (7.4%)	71/143 (49.7%)	<0.0001	6.7 (5.1–8.9)
≥ 3 lesions of maternal vascular malperfusion	11/944 (1.2%)	48/143 (33.6%)	<0.0001	28.8 (15.3–54.1)
Fetal vascular malperfusion	186/944 (19.7%)	126/143 (88.1%)	<0.0001	4.5 (3.9–5.2)
Villous changes	107/944 (11.3%)	122/143 (85.3%)	<0.0001	7.5 (6.2–9.1)
Villous stromal-vascular karyorrhexis	24/944 (2.5%)	63/143 (44.1%)	<0.0001	17.3 (11.2–26.8)
Hyalinized avascular villi, small foci	62/944 (6.6%)	8/143 (5.6%)	0.82	0.9 (0.4–1.7)
Hyalinized avascular villi, variable sized foci	19/944 (2%)	31/143 (21.7%)	<0.0001	10.8 (6.3–18.5)
Fetal thrombotic vasculopathy	2/944 (0.2%)	20/143 (14.0%)	<0.0001	66
Vascular lesions	85/944 (9%)	40/143 (28.0%)	<0.0001	3.1 (2.2–4.3)
Thrombi in large fetal vessels	25/944 (2.6%)	16/143 (11.2%)	<0.0001	4.2 (2.3–7.7)
Intimal fibrin deposition, large fetal vessels	61/944 (6.5%)	27/143 (18.9%)	<0.0001	2.9 (1.9–4.4)
≥ 2 lesions of fetal vascular malperfusion	7/944 (0.7%)	36/143 (25.2%)	<0.0001	34 (15.4–74.8)

Table 5 (continued)

Histologic lesions	Normal pregnancy (n = 944)	Fetal death (n = 143)	q-Value	PR
Miscellaneous lesions (hypoxic histologic patterns of placental injury)	82/942 (9%)	85/143 (59%)	<0.0001	6.8 (5.3–8.7)
Nucleated red blood cells	0/942 (0%)	36/143 (25%)	<0.0001	–
Hypercapillarized villi	33/938 (4%)	24/140 (17%)	<0.0001	4.9 (3–8)
Intravillous hemorrhage	1/944 (0.1%)	10/143 (7.0%)	<0.0001	66
Massive perivillous fibrinoid deposition	2/942 (0.2%)	4/143 (3%)	0.006	13.2
Laminar necrosis of the decidua capsularis	52/942 (6%)	38/143 (27%)	<0.0001	4.8 (3.3–7)

Data are presented as number (%). PR, prevalence ratio. ^aSevere chronic inflammatory lesions defined as high-grade lesions of chronic villitis of unknown etiology or grade 2 chronic chorioamnionitis.

Table 6: Cases of fetal death without significant placental pathologic lesions.

Gravida	Body mass index	Number of live births	Gender of fetus	Gestational age at delivery	Baby weight	Placenta weight	Feto-placental weight ratio	Birth weight percentile
4	28.9	3	Male	21.3	359	129	2.78	25 th
6	25.1	3	Female	20.7	93	110	0.84	1 st
5	28.5	3	Female	25	652	178	3.66	27 th
11	21.9	2	Male	24.1	312	151	2.06	1 st
4	47.5	2	Male	20.6	220	98	2.24	1 st
3	56.6	2	Male	32.6	2380	483	4.92	57 th
9	28.1	6	Male	22.3	587	224	2.62	61 st
1	42.3	0	Male	27.1	1055	371	2.84	51 st
1	25	0	Male	21.4	458	200	2.29	53 rd
1	32.1	0	Male	26.7	989	222	4.45	58 th
9	27.5	6	Male	20.7	306	108	2.83	19 th

Among these cases, only 2 (18%; 2/11) did not have any recognized risk factor associated with fetal death. Both cases were male neonates with a birthweight of 359 g at 21.3 weeks (25th percentile) and 306 g at 20.7 weeks (19th percentile), respectively, and their mothers were overweight (body mass indices of 28.9 kg/m² and 27.5 kg/m²) and multiparous (one with three prior live births and the other with six prior live births).

[Correction added after online publication 22 April 2020: symbol “<” was deleted before percentile information (25th, 19th)]

Discussion

Principal findings of the study

The most prevalent placental histopathologic lesions in structurally normal stillbirths as compared to controls included disorders of villous maturation, histologic patterns suggestive of hypoxia, fetal vascular malperfusion, maternal vascular malperfusion, and chronic placental inflammation.

In addition to the placental histologic lesions described above, preterm birth, fetoplacental weight ratio <10th percentile for gestational age, marginal umbilical cord insertion, and hypocoiled umbilical cord were more frequently found in the cases of fetal death.

Disorders of villous maturation

Disorders of villous maturation [delayed villous maturation, 22% (31/143); accelerated villous maturation, 20% (28/143); and maturation arrest, 4% (5/143)] were all significantly higher in the fetal death cases as compared to the controls: 1% (4/405), 0% (0/405), and 0.1% (1/942), respectively (Figure 2). These findings are consistent with a previous report from our group [104] in which delayed villous maturation was reported in 20.7% (12/58) and accelerated villous maturation in 15.5% (9/58) of placentas of fetal death, both percentages significantly higher than that of the controls, 5% (3/60) and 0% (0/60) [104]. Moreover, accelerated villous maturation was associated with high burden maternal vascular malperfusion lesions (defined as two or more lesions of maternal vascular malperfusion) than that of fetal death cases without maturation disorders but with maternal

vascular malperfusion lesions [82% (23/28) vs. 28% (22/80), $q < 0.0001$].

Both delayed villous maturation and accelerated villous maturation have been reported in only two other previous studies [52, 53]. Korteweg et al. found delayed villous maturation in 4.3% and accelerated villous maturation in 12% of their stillbirth cases, although the terminology used to describe these lesions was villous immaturity and hypoplasia, respectively [52]. Gibbins et al. reported delayed villous maturation in 9.9% and accelerated villous maturation in 7.4% of fetal death cases [53]. Only delayed villous maturation (terminal villous immaturity) has been reported by Bukowski et al. [44] and Pinar et al. [46] in 10.1% vs. 2.3% and 10.3% vs. 2.3%, respectively, in fetal death cases compared to the controls.

It is noteworthy that there is considerable variability in the definition of villous maturational disorders [105–108]. The assessment of the presence of delayed villous maturation in the placenta is subjective, with poor concordance among pathologists and prone to much inter-observer variability [104, 109]. However, the diagnosis of delayed villous maturation in our study was based on CD15 staining, a diagnostic marker of persistent villous immaturity and chronic placental dysfunction, which enhances an objective interpretation of this disorder of villous maturation [58, 59, 104, 110, 111]. Endothelial immunophenotypic transformation with significant elevation in immature CD15⁺ endothelial cells in the macro-vasculature (chorionic plate and stem villous vessels) and micro-vasculature (terminal villous vessels) is diagnostic for delayed villous maturation [58, 104].

Similarly, subjective differences in the application of diagnostic criteria for accelerated villous maturation hinder reproducibility. It is known that the distinction between accelerated villous maturation and distal villous hypoplasia, both associated with maternal vascular malperfusion, needs clarification and that the pattern of accelerated villous maturation is not uniform, thus making reproducibility a challenge [57, 105, 112, 113]. Currently, there are no immunohistochemical markers to render the diagnosis of accelerated villous maturation objective.

Maturation arrest was observed in 4% (5/143) of the fetal death cases as compared to the controls [0.1% (1/942)]. *The lesion was observed in approximately 30% of villous parenchyma.* This entity has not been reported in studies on fetal death. The terminology of villous maturation arrest [39, 114] has not been uniform in the literature, where it has also been referred to as persisting immaturity [43, 115] and is considered to be a villous maturation disorder [38, 39, 43, 116]. A placenta with maturation arrest displays primitive mesenchymal, embryonic, and loose

reticular stroma with Hofbauer cells [38, 39]. Capillaries are few and exhibit only rare contact with the surface epithelium, and so there are considerably reduced vasculo-syncytial membranes and the stem villi are narrow with reduced vascular α -smooth muscle actin positive cells [38, 39]. Immature intermediate villi may cause considerable difficulty in histologic differentiation from villous edema. This complexity is evident because the reticular stromal core of immature intermediate villi has a weak affinity for conventional stains, given the lack of collagen, and may impart a histologic picture of seemingly edematous villi with accumulated interstitial fluid [34].

What is the significance of disorders of villous maturation in fetal death?

The hallmark of delayed villous maturation is insufficient development of the terminal villi, reduced vascularization of the chorionic villi, and lack of vasculo-syncytial membranes [32, 34]. Normal development of vasculo-syncytial membranes shortens the diffusion distance between maternal and fetal circulation, thereby increasing oxygen transport at the third power [117]. Normal terminal villi show two to three vasculo-syncytial membranes per terminal villus [31, 42, 118], and from gestational week 10–40, placental oxygen diffusion capacity increases 30-fold [117]. Increased thickness of the placental barrier in term placentas with delayed villous maturation or structural immaturity for gestational age contributes to fetal hypoxia and places the fetus at risk, including fetal death [31, 32, 40, 119, 120]. We have recently published that placental delayed villous maturation is associated with evidence of chronic fetal hypoxia (manuscript in press).

Proposed pathogenesis of accelerated villous maturation is defective remodeling of the spiral arteries [35, 62, 121], which leads to malperfusion of the placenta, causing loss of function through oxidative and endoplasmic reticulum stress, decreased surface area, damage to the syncytiotrophoblast, and hypoxemia [122]. This results in altered development of the fetal organs, increased risk of fetal growth restriction, preterm delivery, brain injury, and fetal death [123–129], while maternal conditions include recurrence in future pregnancies, hypertension, preeclampsia, diabetes with arteriopathy, and autoimmune conditions [130].

The current study demonstrates that placentas of fetal death were 44 times more likely to present disorders of villous maturation compared to placentas of normal pregnancy.

Placental histologic patterns suggestive of hypoxia

Lesions demonstrating histologic patterns suggestive of hypoxia included nucleated red blood cells [34, 131–133], hypercapillarized villi [34, 64, 134, 135], intravillous hemorrhage [65, 66], massive perivillous fibrinoid deposition [64, 88, 136–141], and laminar necrosis of the decidua capsularis [68, 142].

Nucleated red blood cells in stillbirth has been previously reported [143, 144]. In the current study, nucleated red blood cells were observed in 25% (36/143) of fetal death cases but not in the control placentas with a normal pregnancy outcome [0% (0/942)]. The gestational age ranged from 21.7 weeks to 38.6 weeks. Nucleated red blood cells in the current study showed highest association with delayed villous maturation [33% (12/36)] and high burden maternal vascular malperfusion lesions [three or more lesions: 28% (10/36)].

Histologic studies to quantify nucleated red blood cells are few and have been performed near term [145, 146]. Pertinently, pathologists evaluating placentas do not routinely assess for increased nucleated red blood cells within placental vessels, as pointed out in a study that reported that 38% pathologists did not routinely assess for nucleated red cells [147]. Moreover, in normal preterm infants, nucleated red blood cell values are more difficult to interpret and are confounded by the etiology of the preterm delivery [145, 148].

The methods described to quantify nucleated red blood cells in placenta vary. Maier et al. reported that placental examination may provide information about fetal hypoxia [149]. Only recently has it been found feasible to calculate nucleated red blood cell counts from histologic slides of the placenta [145]. Reporting nucleated red blood cells as a ratio per 100 white blood cells has been found wanting since white blood cell counts in newborn are variable and that nucleated red blood cell counts could be misleadingly low or high if reported relative to white cells [148, 150]. Therefore, in our study, a less quantitative approach to count nucleated red blood cells was utilized [146]. Nucleated red blood cells were counted in sections using high power objective (40×) and ten fields were observed. A count of greater than ten nucleated red blood cells in ten high-power fields (HPFs) was considered abnormal. Redline has established that a value of greater than ten nucleated red blood cells per 10 HPFs corroborates with an absolute value of $2.5 \times 10^3/\text{mm}^3$ or more with high sensitivity and specificity and is considered as an elevated nucleated red cell count [146]. Although nucleated red blood cells were observed in our study in a

wide gestational age range, only those cases that showed greater than ten nucleated red blood cells per 10 HPFs were considered pathological.

Fetal hypoxemia is understood to be the major underlying factor leading to the increased production and premature release of immature erythrocyte precursors, by stimulating increased production and secretion of erythropoietin [131–133, 148, 151–154]. Since erythropoietin does not cross the placenta and is not stored, increased fetal plasma and amniotic fluid levels are considered to represent true fetal hypoxemia [133, 155]. We have recently demonstrated that delayed villous maturation is associated with higher concentration of amniotic fluid erythropoietin (manuscript submitted).

Hypercapillarization was observed in 17% (24/140) of placentas with fetal death as compared to 4% (33/938) of placentas with normal pregnancy outcome. The prevalence ratio of this lesion was five times higher than that in normal pregnancy. Notably, 54% (13/24) of these cases were associated with retroplacental hemorrhage (placental abruption). Hypercapillarization has not been reported in previous stillbirth studies.

Hypercapillarization has been proposed to be the result of long-term exposure of the placenta to hypoxic conditions [156, 157] and generally assumed to result from impaired uteroplacental blood flow [88, 135]. This process is expressed as capillary profiles per villous profile [134]. Studies by Kumazaki et al. demonstrated that vascular endothelial growth factor (VEGF) and kinase insert domain-containing receptor (KDR) in placentas (VEGF/KDR) induce effective angiogenesis, resulting in villous hypercapillarization under mild and persistent hypoxia/ischemia [158]. Their findings suggest that a paracrine stimulation of endothelial cells by VEGF via the KDR receptor is enhanced within the villous blood vessels in response to hypoxia/ischemia [158].

Intravillous hemorrhage, chorionic villous hemorrhage, or villous stromal hemorrhage was observed in 7.0% (10/143) of the placentas with fetal death as compared to none in the normal outcome group [0% (0/944)]. The prevalence ratio of this lesion was 66 times higher in cases of fetal death than in normal pregnancy. Similar to hypercapillarization, it is noteworthy that 80% (8/10) of the cases with intravillous hemorrhage were associated with retroplacental hemorrhage (placental abruption). Although Mooney et al. reported the association of intravillous hemorrhage with retroplacental hemorrhage in 51% (31/60) of their cases [65], this is the first study to report intravillous hemorrhage in placentas of fetal death.

Intravillous hemorrhage was first described by Mooney et al. as hemorrhage involving the chorionic villi with the rupture of villous vessels and the extravasation of red blood cells in the stroma [65]. This lesion primarily involves terminal villi and needs to be differentiated from villous stromal vascular karyorrhexis that affects stem and intermediate villi and is characterized by the presence of intact and fragmented red blood cells within the vascular wall and lumen [66]. Intravillous hemorrhage is considered to be a manifestation of an acute hypoxic event of the placenta [159] consequence of hypoxic necrosis of the villous vessel with subsequent loss of vascular integrity and stromal hemorrhage [65].

Massive perivillous fibrinoid deposition of the placenta or maternal floor infarction was observed in 3% (4/143) of the fetal death cases as compared to 0.2% (2/942) of the normal pregnancy controls. The prevalence ratio of this lesion was 13 times higher than that of normal controls. This lesion has been reported in stillbirth placentas by other investigators with a frequency ranging between 0.9% and 10% [52, 53, 128, 160, 161].

Massive perivillous fibrinoid deposition was first described in 1967 [162] and is associated with recurrent serious adverse pregnancy outcomes [64, 88, 136–138] that may include miscarriage [70, 136, 139, 163], fetal growth restriction [70, 136, 139, 140, 164–169], and fetal death [136, 139–141, 165, 168, 170, 171]. The amorphous material present in this condition appears to be a combination of trophoblast-derived extracellular substance (matrix-predominant type fibrinoid) and serum fibrin of the maternal coagulation cascade (laminated fibrin-type fibrinoid) [70, 89, 137, 167, 169, 172]. It has been suggested that hypoxic trophoblastic or villous damage could result in abnormal activation of coagulation in the intervillous space, potentiating an otherwise low-grade fibrinoid deposition [64, 89, 138]. The main differential diagnosis for massive perivillous fibrinoid deposition is chorionic villous infarction, cotyledon infarct, and gitterinfarct [36, 173]. Chorionic villous infarct is a circumscribed area of parenchymal necrosis (Table 1). Cotyledon infarct is parenchymal necrosis limited to one cotyledon [36]. Gitterinfarcts, on the other hand, are described as matting together of chorionic villi by fibrinoid material which macroscopically appear as grey-white firm irregular lesions [36, 138]. Gitterinfarcts are clinically related to materno-placental circulatory disturbances and maternal thrombophilia [36].

Laminar necrosis of the decidua capsularis (placental membranes) was observed in 27% (38/143) of placentas with fetal death as compared to 6% (52/942) in the normal

pregnancy controls. The prevalence ratio of this lesion was five times higher than that of the control group. Laminar necrosis was observed in 34% (13/38) of fetal death cases with lesions of maternal vascular malperfusion.

Laminar necrosis is a histologic lesion of unclear pathogenesis characterized by a band of coagulative necrosis at the choriodecidual interphase [68]. This lesion has been reported in placentas affected by preeclampsia, preterm prelabor rupture of the fetal membranes, preterm abruption [68], fetal growth restriction [174], maternal hypertensive disorders, and other conditions linked to *in utero* hypoxia [68, 142]. Based on immunohistochemical studies, it has been suggested that laminar necrosis be recognized as a hypoxic placental lesion [68]. The current study is the first to report laminar necrosis in association with fetal death.

Fetal vascular malperfusion lesions

The frequency of lesions of fetal vascular malperfusion (both villous and vascular) was higher in cases of fetal death than in the controls. Although previous studies have reported the frequency of lesions of fetal vascular malperfusion up to 72.8% [44, 46, 48, 51–53, 128, 160, 175–177], the issue of differentiation of true fetal vascular malperfusion from stillbirth-induced changes has been addressed only by our group in a previous study [104].

Given that maceration and stillbirth-related intrauterine regressive placental changes are useful in understanding the timing of fetal death [178], they obscure the interpretation of placental pathologic findings [178, 179]. Thus, histologic changes observed in lesions of fetal vascular malperfusion, such as villous-stromal-vascular karyorrhexis, clusters of sclerotic/avascular chorionic villi, and stem villous vessel obliteration, can all be observed in placentas of fetal death and are a result of a cessation of blood flow through the fetal vessels [57, 91, 180]. Indeed, 85% (74/87) of cases of fetal death in the current study showed maceration with diffuse placental histologic changes and an absence of well-circumscribed avascular villi co-existing with normally perfused villi. Moreover, these cases did not show layered strata of fibrin, leukocytes, and red cells, which are pathognomonic of thrombi in flowing blood or vascular ectasia in the muscularized vessels of the chorionic plate and/or stem villi to suggest subacute and chronic restriction of umbilical blood flow [91, 181, 182].

The duration of fetal death before delivery in the current study was considered to be more than 48 h based on clinical evidence of life before delivery as well as gross morphology of the fetus (maceration) and placental histologic confirmation, according to established criteria [178,

183–185]. It is known that it takes approximately 2 days for the early changes of fetal vascular malperfusion to be observed in villi [178, 185]. Given that fetal vascular malperfusion changes identified in the cases of the current study did not occur within the 48-h interval between fetal death and delivery, these lesions were considered as post-mortem changes and not reflective of true pathologic fetal vascular malperfusion lesions contributing to fetal death [91]. Moreover, the most common etiology of fetal vascular malperfusion is umbilical cord obstruction leading to stasis, ischemia, and, in some cases, thrombosis, mechanisms that include extrinsic pressure (e.g. umbilical cord entanglements, umbilical cord prolapse), intrinsic deformation (e.g. hypercoiling, torsion), and increased resistance to blood flow (e.g. excessive length of the umbilical cord) [94, 95, 186]. Except for marginal umbilical cord insertion, none of the aforementioned risk factors, which could obstruct the umbilical blood flow, showed any significant association between cases and controls.

Pertinently, Garrod et al. reported significant changes in placental histology such as reduction in the number of blood vessels per villus, an increased frequency of distal villous hypoplasia, as well as avascular villi in all of the stored placentas (formalin storage/refrigeration) after 72 h, but there were no significant changes in the frequency of infarction, excessive syncytial knots, or villous immaturity [187]. This important observation, which is consistent with our findings, suggests that placental lesions of maternal vascular malperfusion (infarction, increased syncytial knots) and delayed villous maturation are not artifacts secondary to fetal death.

The aforementioned study raises an important concern that morphologic changes in the placenta should reflect pathologies and not artifacts induced by the duration of placental storage – specifically, lesions described after fetal death, such as fetal vascular malperfusion [181, 182, 187]. The presence of stromal villous-vascular karyorrhexis, avascular villi, and stem villous vessel obliteration was noted in the current study; however, they were considered as postmortem changes secondary to cessation of the fetal circulation [104, 178]. Previous studies on stillbirth reporting a high frequency of lesions of fetal vascular malperfusion have failed to address this important issue except for one study [188].

Maternal vascular malperfusion lesions

The frequency of maternal vascular malperfusion lesions (both villous and vascular changes) was significantly higher in the cases of fetal death than in the controls

[75.5% (108/143) vs. 35.7% (337/944); $P < 0.0001$; prevalence ratio, 2.1 (1.9–2.4)]. These types of lesions reported in previous publications have ranged from 7% to 82% [44, 45, 52, 53, 128, 160, 176, 189]. Our findings are consistent with Man et al. [160], who observed lesions of maternal vascular malperfusion in 70% (42/60) of stillbirth cases. Similarly, the frequency of villous infarcts [35% (50/143)] corresponds with a case-control study of stillbirth and live birth reported by the Stillbirth Collaborative Research Network [53], which indicated villous infarcts in 25.6% of the cases.

Amir et al. reported that over 90% of preterm (<37 weeks) and over 83% of term fetal death placentas were associated with lesions consistent with uteroplacental insufficiency [176]. However, the author's definition of uteroplacental insufficiency included a wide range of lesions, such as placental infarcts, poor vascularity of the chorionic villi, intravascular thrombi, and vascular occlusion [176]. Some of these lesions may not reflect uteroplacental insufficiency [93].

Gibbins et al. [128] reported an overwhelming presence of lesions of maternal vascular malperfusion in cases of fetal death. However, cases were defined as fetal death with preeclampsia/gestational hypertension vs. stillbirths without preeclampsia/gestational hypertension; and the secondary comparison was stillbirths with preeclampsia/gestational hypertension vs. live births with preeclampsia/gestational hypertension [128]. Preeclampsia is known to be associated with placental lesions of maternal vascular malperfusion [190–193]; our findings are consistent with this.

Chronic placental inflammatory lesions

Cases of fetal death exhibited a higher frequency of chronic inflammatory lesions than the controls – specifically, chronic chorioamnionitis grade 1/stage 1, chronic villitis of infectious origin, and chronic deciduitis. Previous reports of chronic inflammatory lesions in fetal death have reported a frequency between 0.7% and 57.9% [48, 49, 51, 52, 160, 161, 177, 194, 195].

Since a major immunological function of the placenta is the successful establishment and maintenance of maternal-fetal tolerance [196–204], breakdown in tolerance has been implicated in maternal anti-fetal rejection. The pathologic lesions associated with maternal anti-fetal rejection include chronic chorioamnionitis [18, 96, 97, 205–207], villitis of unknown etiology [96, 98, 205, 208], chronic deciduitis [96, 205, 207], and massive perivillous fibrin deposition [195, 209].

Is villitis of unknown etiology less frequent in cases of fetal death?

Herein, the frequency of villitis of unknown etiology was significantly lower in the cases of fetal death than in the controls [10.5% (15/143) vs. 18.5% (175/944), $q < 0.02$] (Table 4). The higher rate of villitis of unknown etiology in the placentas of women with a normal pregnancy is unlikely to be due to an infectious agent and favors an immunological mechanism to explain these findings [56]. An immunological stand-off between the mother and fetus occurs at the maternal-fetal interface throughout normal pregnancy, and the presence of maternal lymphocytes infiltrating the interface would represent maternal-fetal cellular interaction without inevitably indicating a pathologic process [56]. In all except two cases (13/15) of fetal death with villitis of unknown etiology in the current study, the patients were in the third trimester of pregnancy; the two patients were in the second trimester of pregnancy, associated with massive perivillous fibrinoid deposition. Our findings are consistent with the observations of Redline [210], who reported that villitis of unknown etiology is typically observed in the third trimester and, in particular, in placentas from term or near-term pregnancies [210, 211].

Acute placental inflammatory lesions

The frequency of acute placental inflammatory lesions in cases of fetal death has varied between 4.9% and 76% [44–46, 48–51, 175, 177, 194, 212–215] (Table 4).

Although the frequency of acute inflammatory response in the chorioamniotic membranes (maternal) and acute fetal inflammatory response did not differ from the controls, the frequency of severe (high stage and high grade) acute chorioamnionitis (maternal) was significantly higher in the cases of fetal death than in the controls. Notably, the frequency of low-stage and low-grade fetal inflammatory response (chorionic vasculitis or umbilical phlebitis) was significantly lower in the cases of fetal death than in the controls and fetal inflammatory response was four times less frequent than maternal inflammatory response in fetal deaths.

Conventionally, acute chorioamnionitis, funisitis, and chorionic vasculitis have been considered as manifestations of the amniotic fluid infection syndrome, but it is now recognized that these lesions can occur in the absence of bacteria or viruses in the amniotic cavity and that these processes can be attributed to danger signals, or alarmins, that can initiate an inflammatory response in

the absence of microbial products [216–220]. Moreover, it is established that labor *per se* is an inflammatory state associated with an infiltration of inflammatory cells into the myometrium and chorioamniotic membranes [195, 221]. Some patients may develop acute inflammatory lesions and, specifically, acute chorioamnionitis during the induction of labor indicated because of fetal death [195].

We have reported earlier that intrauterine inflammation is present in one-fifth of cases of intrauterine fetal death and that a fetal inflammatory response (i.e. funisitis) was seven times less frequent than a maternal inflammatory response (i.e. histological chorioamnionitis) [17]. A possible explanation for this is that fetal infection occurs but fails to trigger a fetal inflammatory response and the onset of preterm labor [17] and fetal death would represent failure of the host response mechanisms dealing with intrauterine infection [17]. Another explanation for the discrepant rate of fetal and maternal inflammatory responses is that histological chorioamnionitis may occur after fetal death and is etiologically unrelated to the fetal demise [17].

It is also known that the administration of an agent such as a prostaglandin [222–230], insertion of a Foley catheter [231], or other method of induction of labor may predispose to inflammation.

Placental pathology findings in the context of previous studies

Although placental pathology in stillbirth has been reported in previous publications [31, 44–53, 71, 128, 160, 161, 175–177, 188, 189, 194, 213–215, 232–246] (Supplementary Table 1), our study is the first to systematically evaluate the placenta in fetal death according to (1) recommendations of the Amsterdam Placental Workshop Group; (2) utilization of immunohistochemical techniques; (3) description of disorders of villous maturation in stillbirth; (4) description of hypoxic histologic patterns of placental injury; (5) description of chronic placental inflammatory lesions; (6) comparison of placental findings in stillbirth to placentas from normal term controls; and (7) evaluation of the placenta and its histology by trained perinatal pathologists.

Previous studies have lacked standardized nomenclature, definitions, and subcategorization of placental lesions [49, 50, 177, 214, 234, 235] as outlined by the Amsterdam Placental Workshop Group [57] or the classifications have been the authors' own or their modifications of existing terminologies [31, 44, 46, 47, 49, 50, 53,

128, 160, 161, 176, 177, 213, 214, 232, 233, 235]. Moreover, the study designs of previous reports have varied, with most being cases of stillbirth lacking adequate term live birth controls [49, 51, 52, 160, 161, 175–177, 194, 213, 214, 232–235]. Live birth controls in some reports were preterm [46], which by definition are abnormal and may not be representative of all fetuses alive at the given gestational age [46]. In addition, other researchers compared cases of stillbirth in patients with preeclampsia/gestational hypertension to stillbirths in patients without these medical conditions [128]. The importance of placental evaluation by trained perinatal pathologists has been highlighted by investigators who have shown that the discrepancies in placental pathologic diagnoses were the result of a lack of experience of general surgical pathologists [173]. Supplementary Table 1 shows all the placental studies on stillbirth.

Strengths and limitations

The strengths of this study encompass (1) the large number of patients; (2) the standardized and comprehensive placental examination performed by placental pathologists; (3) the usage of immunohistochemistry to confirm delayed villous maturation; (4) the adaption of standardized nomenclature recommended by the Amsterdam Placental Workshop Group; and (5) the comparison of placental findings in stillbirth to placentas from normal term controls.

A limitation of this study is that by design, it is not possible to draw inferences about causation between the presence of a particular placental lesion and fetal death. Another limitation is that the control group consisted of patients who delivered normal pregnancies at term, rather than a group of gestational age-matched pregnancies. We did not choose the latter because by definition, all preterm deliveries are the result of a pathologic process. A group of patients who delivered preterm with normal pregnancies would be very difficult to obtain.

Conclusion

Disorders of villous maturation are forty-four times more likely to present in cases of fetal death suggesting a substantial burden of this lesion in fetal death.

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References

1. Blencowe H, Cousens S, Jassir FB, Say L, Chou D, Mathers C, et al. National, regional, and worldwide estimates of stillbirth rates in 2015, with trends from 2000: a systematic analysis. *Lancet Glob Health* 2016;4:e98–108.
2. MacDorman MF, Gregory EC. Fetal and perinatal mortality: United States, 2013. *Natl Vital Stat Rep* 2015;64:1–24.
3. Willinger M, Ko CW, Reddy UM. Racial disparities in stillbirth risk across gestation in the United States. *Am J Obstet Gynecol* 2009;201:469.e1–8.
4. Smulian JC, Ananth CV, Vintzileos AM, Scorza WE, Knuppel RA. Fetal deaths in the United States. Influence of high-risk conditions and implications for management. *Obstet Gynecol* 2002;100:1183–9.
5. Allen VM, Joseph K, Murphy KE, Magee LA, Ohlsson A. The effect of hypertensive disorders in pregnancy on small for gestational age and stillbirth: a population based study. *BMC Pregnancy Childbirth* 2004;4:17.
6. Aagaard-Tillery KM, Holmgren C, Lacoursiere DY, Houssain S, Bloebaum L, Satterfield R, et al. Factors associated with nonanomalous stillbirths: the Utah Stillbirth Database 1992–2002. *Am J Obstet Gynecol* 2006;194:849–54.
7. Ahmad AS, Samuelsen SO. Hypertensive disorders in pregnancy and fetal death at different gestational lengths: a population study of 2 121 371 pregnancies. *Br J Obstet Gynaecol* 2012;119:1521–8.
8. Stormdal Bring H, Hulthen Varli IA, Kublickas M, Papadogiannakis N, Pettersson K. Causes of stillbirth at different gestational ages in singleton pregnancies. *Acta Obstet Gynecol Scand* 2014;93:86–92.
9. Harmon QE, Huang L, Umbach DM, Klungsoyr K, Engel SM, Magnus P, et al. Risk of fetal death with preeclampsia. *Obstet Gynecol* 2015;125:628–35.

10. Panaitescu AM, Syngelaki A, Prodan N, Akolekar R, Nicolaides KH. Chronic hypertension and adverse pregnancy outcome: a cohort study. *Ultrasound Obstet Gynecol* 2017;50:228–35.
11. Rosenstein MG, Cheng YW, Snowden JM, Nicholson JM, Doss AE, Caughey AB. The risk of stillbirth and infant death stratified by gestational age in women with gestational diabetes. *Am J Obstet Gynecol* 2012;206:309.e1–7.
12. Starikov R, Dudley D, Reddy UM. Stillbirth in the pregnancy complicated by diabetes. *Curr Diab Rep* 2015;15:11.
13. McClure EM, Goldenberg RL. Infection and stillbirth. *Semin Fetal Neonatal Med* 2009;14:182–9.
14. Goldenberg RL, McClure EM, Saleem S, Reddy UM. Infection-related stillbirths. *Lancet* 2010;375:1482–90.
15. Wijis LA, de Graaff EC, Leemaqz S, Dekker G. Causes of stillbirth in a socioeconomically disadvantaged urban Australian population – a comprehensive analysis. *J Matern Fetal Neonatal Med* 2017;30:2851–7.
16. Blackwell S, Romero R, Chaiworapongsa T, Refuerzo J, Gervasi MT, Yoshimatsu J, et al. Unexplained fetal death is associated with changes in the adaptive limb of the maternal immune response consistent with prior antigenic exposure. *J Matern Fetal Neonatal Med* 2003;14:241–6.
17. Blackwell S, Romero R, Chaiworapongsa T, Kim YM, Bujold E, Espinoza J, et al. Maternal and fetal inflammatory responses in unexplained fetal death. *J Matern Fetal Neonatal Med* 2003;14:151–7.
18. Lee J, Romero R, Dong Z, Xu Y, Qureshi F, Jacques S, et al. Unexplained fetal death has a biological signature of maternal anti-fetal rejection: chronic chorioamnionitis and alloimmune anti-human leucocyte antigen antibodies. *Histopathology* 2011;59:928–38.
19. Richani K, Romero R, Soto E, Espinoza J, Nien JK, Chaiworapongsa T, et al. Unexplained intrauterine fetal death is accompanied by activation of complement. *J Perinat Med* 2005;33:296–305.
20. Ananth CV, Berkowitz GS, Savitz DA, Lapinski RH. Placental abruption and adverse perinatal outcomes. *J Am Med Assoc* 1999;282:1646–51.
21. Ananth CV, Wilcox AJ. Placental abruption and perinatal mortality in the United States. *Am J Epidemiol* 2001;153:332–7.
22. Raisanen S, Gissler M, Nielsen HS, Kramer MR, Williams MA, Heinonen S. Social disparity affects the incidence of placental abruption among multiparous but not nulliparous women: a register-based analysis of 1,162,126 singleton births. *Eur J Obstet Gynecol Reprod Biol* 2013;171:246–51.
23. Stanek J. Placental examination in nonmacerated stillbirth versus neonatal mortality. *J Perinat Med* 2018;46:323–31.
24. Nkwabong E, Tiomela Goula G. Placenta abruption surface and perinatal outcome. *J Matern Fetal Neonatal Med* 2017;30:1456–9.
25. Korteweg FJ, Erwich JJ, Timmer A, van der Meer J, Ravise JM, Veeger NJ, et al. Evaluation of 1025 fetal deaths: proposed diagnostic workup. *Am J Obstet Gynecol* 2012;206:53.e1–12.
26. Committee on Genetics. ACOG Committee Opinion No. 383: evaluation of stillbirths and neonatal deaths. *Obstet Gynecol* 2007;110:963–6.
27. Pinar H, Koch MA, Hawkins H, Heim-Hall J, Shehata B, Thorsten VR, et al. The Stillbirth Collaborative Research Network (SCRN) placental and umbilical cord examination protocol. *Am J Perinatol* 2011;28:781–92.
28. Flenady V, Middleton P, Smith GC, Duke W, Erwich JJ, Khong TY, et al. Stillbirths: the way forward in high-income countries. *Lancet* 2011;377:1703–17.
29. Flenady V, Wojcieszek AM, Middleton P, Ellwood D, Erwich JJ, Coory M, et al. Stillbirths: recall to action in high-income countries. *Lancet* 2016;387:691–702.
30. Benirschke K, Kaufmann P. Three-dimensional aspects of villous maldevelopment. In: Benirschke K, Kaufmann P, editors. *Pathology of the human placenta*. 2nd ed. New York: Springer-Verlag; 1990. p. 114–29.
31. Stallmach T, Hebisch G, Meier K, Dudenhausen JW, Vogel M. Rescue by birth: defective placental maturation and late fetal mortality. *Obstet Gynecol* 2001;97:505–9.
32. Gordijn SJ, Korteweg FJ, Erwich JJHM, Holm JP, Ravise JM, Nikkels PGJ, et al. Placental villus immaturity as an important cause of term fetal death. In: Gordijn SJ, editor. *On perinatal pathology: aspects of perinatal autopsy, placental pathology and classification of perinatal mortality*. Groningen: University of Groningen; 2009.
33. Kraus FT, Redline RW, Gersell DJ, Nelson DM, Dicke JM. *AFIP atlas of nontumor pathology: placental pathology*. 1st ed. Washington, DC: American Registry of Pathology in collaboration with the Armed Forces Institute of Pathology; 2004.
34. Benirschke K, Kaufmann P, Baergen RN. *Pathology of the human placenta*. 5th ed. New York: Springer-Verlag New York; 2006.
35. Khong TY, Mooney E, Nikkels PGJ, Morgan TK, Gordijn SJ, editors. *Pathology of the placenta: a practical guide*. Switzerland: Springer Nature; 2019.
36. Vogel M, Turowski G. *Clinical pathology of the placenta*. Berlin/Boston: Walter de Gruyter GmbH; 2019.
37. Vogel M. Pathologie der Schwangerschaft, der Plazenta und des Neugeborenen. In: Remmele W, editor. *Pathologie, Band 3*. Springer, Heidelberg, New York, Tokyo: Springer Publishing; 1984. p. S509–74.
38. Turowski G, Vogel M. Re-view and view on maturation disorders in the placenta. *APMIS* 2018;126:602–12.
39. Vogel M. *Atlas der morphologischen Plazentadiagnostik*. 2nd ed. Berlin: Springer; 1996.
40. Higgins M, McAuliffe FM, Mooney EE. Clinical associations with a placental diagnosis of delayed villous maturation: a retrospective study. *Pediatr Dev Pathol* 2011;14:273–9.
41. Higgins MF, Russell NM, Mooney EE, McAuliffe FM. Clinical and ultrasound features of placental maturation in pre-gestational diabetic pregnancy. *Early Hum Dev* 2012;88:817–21.
42. Treacy A, Higgins M, Kearney JM, McAuliffe F, Mooney EE. Delayed villous maturation of the placenta: quantitative assessment in different cohorts. *Pediatric Dev Pathol* 2013;16:63–6.
43. Schweikhart G, Kaufmann P, Beck T. Morphology of placental villi after premature delivery and its clinical relevance. *Arch Gynecol* 1986;239:101–14.
44. Bukowski R, Hansen NI, Pinar H, Willinger M, Reddy UM, Parker CB, et al. Altered fetal growth, placental abnormalities, and stillbirth. *PLoS One* 2017;12:e0182874.
45. Mecacci F, Serena C, Avagliano L, Cozzolino M, Baroni E, Rambaldi MP, et al. Stillbirths at term: case control study of risk factors, growth status and placental histology. *PLoS One* 2016;11:e0166514.
46. Pinar H, Goldenberg RL, Koch MA, Heim-Hall J, Hawkins HK, Shehata B, et al. Placental findings in singleton stillbirths. *Obstet Gynecol* 2014;123(2 Pt 1):325–36.

47. Helgadottir LB, Turowski G, Skjeldestad FE, Jacobsen AF, Sandset PM, Roald B, et al. Classification of stillbirths and risk factors by cause of death – a case-control study. *Acta Obstet Gynecol Scand* 2013;92:325–33.
48. Bar J, Schreiber L, Ben-Haroush A, Ahmed H, Golan A, Kovo M. The placental vascular component in early and late intrauterine fetal death. *Thromb Res* 2012;130:901–5.
49. Ogunyemi D, Jackson U, Buyske S, Risk A. Clinical and pathologic correlates of stillbirths in a single institution. *Acta Obstet Gynecol Scand* 1998;77:722–8.
50. Hovatta O, Lipasti A, Rapola J, Karjalainen O. Causes of stillbirth: a clinicopathological study of 243 patients. *Br J Obstet Gynaecol* 1983;90:691–6.
51. Manocha A, Ravikumar G, Crasta J. Placenta in intrauterine fetal demise (IUFD): a comprehensive study from a tertiary care hospital. *J Matern Fetal Neonatal Med* 2019;32:3939–47.
52. Korteweg FJ, Erwich JJ, Holm JP, Ravise JM, van der Meer J, Veeger NJ, et al. Diverse placental pathologies as the main causes of fetal death. *Obstet Gynecol* 2009;114:809–17.
53. Gibbins KJ, Pinar H, Reddy UM, Saade GR, Goldenberg RL, Dudley DJ, et al. Findings in stillbirths associated with placental disease. *Am J Perinatol* 2019. doi: 10.1055/s-0039-1688472. [Epub ahead of print].
54. Macdorman MF, Kirmeyer S. The challenge of fetal mortality. *NCHS Data Brief* 2009:1–8.
55. Alexander GR, Kogan MD, Himes JH. 1994–1996 U.S. singleton birth weight percentiles for gestational age by race, Hispanic origin, and gender. *Matern Child Health J* 1999;3:225–31.
56. Romero R, Kim YM, Pacora P, Kim CJ, Benshalom-Tirosh N, Jaiman S, et al. The frequency and type of placental histologic lesions in term pregnancies with normal outcome. *J Perinat Med* 2018;46:613–30.
57. Khong TY, Mooney EE, Ariel I, Balmus NC, Boyd TK, Brundler MA, et al. Sampling and definitions of placental lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch Pathol Lab Med* 2016;140:698–713.
58. Seidmann L, Suhan T, Kamyshanskiy Y, Nevmerzhitskaya A, Gerein V, Kirkpatrick CJ. CD15 – a new marker of pathological villous immaturity of the term placenta. *Placenta* 2014;35:925–31.
59. Seidmann L, Suhan T, Unger R, Gerein V, Kirkpatrick CJ. Transient CD15-positive endothelial phenotype in the human placenta correlates with physiological and pathological fetoplacental immaturity. *Eur J Obstet Gynecol Reprod Biol* 2014;180:172–9.
60. Morgan TK, Tolosa JE, Mele L, Wapner RJ, Spong CY, Sorokin Y, et al. Placental villous hypermaturation is associated with idiopathic preterm birth. *J Matern Fetal Neonatal Med* 2013;26:647–53.
61. Leavey K, Benton SJ, Grynspan D, Bainbridge SA, Morgen EK, Cox BJ. Gene markers of normal villous maturation and their expression in placentas with maturational pathology. *Placenta* 2017;58:52–9.
62. Redline RW, Boyd TK, Roberts DJ. Placental and gestational pathology. Cohen MC, Scheimberg I, editors. Cambridge: Cambridge University Press; 2018.
63. McPherson RA, Pincus MR, editors. In: Henry's clinical diagnosis and management by laboratory methods. 23rd ed. St. Louis, Missouri: Elsevier; 2017.
64. Faye-Petersen OM, Heller DS, Joshi VV. Handbook of placental pathology. Oxford, UK: Taylor & Francis; 2006.
65. Mooney EE, al Shunnar A, O'Regan M, Gillan JE. Chorionic villous haemorrhage is associated with retroplacental haemorrhage. *Br J Obstet Gynaecol* 1994;101:965–9.
66. Aladjem S, Sander CH. Intravillous hemorrhage. *Arch Pathol Lab Med* 1981;105:499–500.
67. Stanek J. Placental hypoxic overlap lesions: a clinicoplacental correlation. *J Obstet Gynaecol Res* 2015;41:358–69.
68. Stanek J, Al-Ahmadie HA. Laminar necrosis of placental membranes: a histologic sign of uteroplacental hypoxia. *Pediatr Dev Pathol* 2005;8:34–42.
69. Bendon RW, Faye-Petersen O, Pavlova Z, Qureshi F, Elder N, Das A, et al. Histologic features of chorioamnion membrane rupture: development of methodology. *Pediatr Pathol Lab Med* 1997;17:27–42.
70. Katzman PJ, Genest DR. Maternal floor infarction and massive perivillous fibrin deposition: histological definitions, association with intrauterine fetal growth restriction, and risk of recurrence. *Pediatr Dev Pathol* 2002;5:159–64.
71. Iwasenko JM, Howard J, Arbuckle S, Graf N, Hall B, Craig ME, et al. Human cytomegalovirus infection is detected frequently in stillbirths and is associated with fetal thrombotic vasculopathy. *J Infect Dis* 2011;203:1526–33.
72. Rawlinson WD, Hall B, Jones CA, Jeffery HE, Arbuckle SM, Graf N, et al. Viruses and other infections in stillbirth: what is the evidence and what should we be doing? *Pathology* 2008;40:149–60.
73. Stegmann BJ, Carey JC. TORCH Infections. Toxoplasmosis, other (syphilis, varicella-zoster, parvovirus B19), rubella, cytomegalovirus (CMV), and herpes infections. *Curr Womens Health Rep* 2002;2:253–8.
74. Syridou G, Spanakis N, Konstantinidou A, Piperaki ET, Kafetzis D, Patsouris E, et al. Detection of cytomegalovirus, parvovirus B19 and herpes simplex viruses in cases of intrauterine fetal death: association with pathological findings. *J Med Virol* 2008;80:1776–82.
75. Opsjon BE, Nordbo SA, Vogt C. Unrecognized viral infections and chromosome abnormalities as a cause of fetal death – examination with fluorescence in situ hybridization, immunohistochemistry and polymerase chain reaction. *APMIS* 2017;125:826–32.
76. Lamont RF, Sobel JD, Vaisbuch E, Kusanovic JP, Mazaki-Tovi S, Kim SK, et al. Parvovirus B19 infection in human pregnancy. *Br J Obstet Gynaecol* 2011;118:175–86.
77. Bittencourt AL, Garcia AG. Pathogenesis and pathology of hematogenous infections of the fetus and newborn. *Pediatr Pathol Mol Med* 2002;21:353–99.
78. Brown KE, Green SW, Antunez de Mayolo J, Bellanti JA, Smith SD, Smith TJ, et al. Congenital anaemia after transplacental B19 parvovirus infection. *Lancet* 1994;343:895–6.
79. Brown KE, Hibbs JR, Gallinella G, Anderson SM, Lehman ED, McCarthy P, et al. Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). *N Engl J Med* 1994;330:1192–6.
80. Brown KE, Young NS, Liu JM. Molecular, cellular and clinical aspects of parvovirus B19 infection. *Crit Rev Oncol Hematol* 1994;16:1–31.
81. de Krijger RR, van Elsacker-Niele AM, Mulder-Stapel A, Salimans MM, Dreef E, Weiland HT, et al. Detection of parvovirus B19 infection in first and second trimester fetal loss. *Pediatr Pathol Lab Med* 1998;18:23–34.

82. Porter HJ, Khong TY, Evans MF, Chan VT, Fleming KA. Parvovirus as a cause of hydrops fetalis: detection by in situ DNA hybridisation. *J Clin Pathol* 1988;41:381–3.
83. Porter HJ, Quantrell AM, Fleming KA. B19 parvovirus infection of myocardial cells. *Lancet* 1988;1:535–6.
84. Khong TY, Malcomson RDG, editors. *Keeling's fetal and neonatal pathology*. Switzerland AG: Springer International Publishing; 2015. p. XVII, 882.
85. Ernst LM. Maternal vascular malperfusion of the placental bed. *APMIS* 2018;126:551–60.
86. Redline RW, Boyd T, Campbell V, Hyde S, Kaplan C, Khong TY, et al. Maternal vascular underperfusion: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol* 2004;7:237–49.
87. Kim YM, Chaemsaitong P, Romero R, Shaman M, Kim CJ, Kim JS, et al. Placental lesions associated with acute atherosclerosis. *J Matern Fetal Neonatal Med* 2015;28:1554–62.
88. Benirschke K, Kaufmann P, Baergen RN. *Pathology of the human placenta*. 5th ed. New York: Springer-Verlag New York; 2006. p. XX, 1050.
89. Baergen RN. *Manual of Benirschke and Kaufmann's pathology of the human placenta*. 1st ed. New York: Springer-Verlag; 2005. p. XIV, 504.
90. Redline RW. Classification of placental lesions. *Am J Obstet Gynecol* 2015;213(4 Suppl):S21–8.
91. Kraus FT. Fetal thrombotic vasculopathy: perinatal stroke, growth restriction, and other sequelae. *Surg Pathol Clin* 2013;6:87–100.
92. Kraus FT, Redline RW, Gersell DJ, Nelson DM, Dicke JM. *Atlas of non-tumor pathology placental pathology*. Washington DC: American Registry of Pathology; 2004.
93. Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation – a workshop report. *Placenta* 2005;26 Suppl A:S114–7.
94. Redline RW, Pappin A. Fetal thrombotic vasculopathy: the clinical significance of extensive avascular villi. *Hum Pathol* 1995;26:80–5.
95. Redline RW, Ravishanker S. Fetal vascular malperfusion, an update. *APMIS* 2018;126:561–9.
96. Kim CJ, Romero R, Chaemsaitong P, Kim JS. Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. *Am J Obstet Gynecol* 2015;213(4 Suppl):S53–69.
97. Ogge G, Romero R, Lee DC, Gotsch F, Than NG, Lee J, et al. Chronic chorioamnionitis displays distinct alterations of the amniotic fluid proteome. *J Pathol* 2011;223:553–65.
98. Kim JS, Romero R, Kim MR, Kim YM, Friel L, Espinoza J, et al. Involvement of Hofbauer cells and maternal T cells in villitis of unknown aetiology. *Histopathology* 2008;52:457–64.
99. Russell P. Inflammatory lesions of the human placenta. III. The histopathology of villitis of unknown aetiology. *Placenta* 1980;1:227–44.
100. Kim CJ, Romero R, Chaemsaitong 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):S29–52.
101. Heerema-McKenney A. Defense and infection of the human placenta. *APMIS* 2018;126:570–88.
102. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Methodol* 1995;57:289–300.
103. R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015. <https://www.R-project.org/>.
104. Pacora P, Romero R, Jaiman S, Erez O, Bhatti G, Panaitescu B, et al. Mechanisms of death in structurally normal stillbirths. *J Perinat Med* 2019;47:222–40.
105. Christians JK, Gynspan D. Placental villous hypermaturation is associated with improved neonatal outcomes. *Placenta* 2019;76:1–5.
106. Huynh J, Dawson D, Roberts D, Bentley-Lewis R. A systematic review of placental pathology in maternal diabetes mellitus. *Placenta* 2015;36:101–14.
107. Evers IM, Nikkels PG, Sikkema JM, Visser GH. Placental pathology in women with type 1 diabetes and in a control group with normal and large-for-gestational-age infants. *Placenta* 2003;24:819–25.
108. Daskalakis G, Marinopoulos S, Krielesi V, Papapanagiotou A, Papantoniou N, Mesogitis S, et al. Placental pathology in women with gestational diabetes. *Acta Obstet Gynecol Scand* 2008;87:403–7.
109. Al-Adnani M, Marnerides A, George S, Nasir A, Weber MA. “Delayed villous maturation” in placental reporting: concordance among consultant pediatric pathologists at a single specialist center. *Pediatr Dev Pathol* 2015;18:375–9.
110. Seidmann L, Anspach L, Roth W. The embryo-placental CD15-positive “vasculogenic zones” as a source of propranolol-sensitive pediatric vascular tumors. *Placenta* 2016;38:93–9.
111. Seidmann L, Kamyschanskiy Y, Martin SZ, Fruth A, Roth W. Immaturity for gestational age of microvasculature and placental barrier in term placentas with high weight. *Eur J Obstet Gynecol Reprod Biol* 2017;215:134–40.
112. Mukherjee A, Chan AD, Keating S, Redline RW, Fritsch MK, Machin GA, et al. The placental distal villous hypoplasia pattern: interobserver agreement and automated fractal dimension as an objective metric. *Pediatr Dev Pathol* 2016;19:31–6.
113. Cui R, Li M, Lu J, Bai H, Zhang Z. Management strategies for patients with placenta accreta spectrum disorders who underwent pregnancy termination in the second trimester: a retrospective study. *BMC Pregnancy Childbirth* 2018;18:298.
114. Becker V. *Allgemeine und Spezielle Pathologie der Plazenta*. In: Becker V, Schiebeler H, Kubli F, editors. *Die Plazenta des Menschen*. Stuttgart, Germany: Thieme Medical Publishers; 1981.
115. Kaufmann P. Architecture of normal villous tree. In: Benirschke K, Kaufmann P, Baergen R, editors. *Pathology of the human placenta*. 2nd ed. Berlin, Heidelberg, New York, Tokyo: Springer Publishing; 1990. p. 81–113.
116. Kaufmann P, Luckhardt M, Schweikhardt G, Cattle SJ. Cross-sectional features and three-dimensional structure of human placental villi. *Placenta* 1987;8:235–47.
117. Mayhew TM, Jackson MR, Boyd PA. Changes in oxygen diffusive conductances of human placentae during gestation (10–41 weeks) are commensurate with the gain in fetal weight. *Placenta* 1993;14:51–61.
118. de Laat MW, van Alderen ED, Franx A, Visser GH, Bots ML, Nikkels PG. The umbilical coiling index in complicated pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2007;130:66–72.
119. Redline RW. Distal villous immaturity. *Diagn Histopathol* 2012;18:189–94.
120. Sankar KD, Bhanu PS, Kiran S, Ramakrishna BA, Shanthi V. Vasculosyncytial membrane in relation to syncytial knots com-

- plicates the placenta in preeclampsia: a histomorphometrical study. *Anat Cell Biol* 2012;45:86–91.
121. Mayhew TM, Barker BL. Villous trophoblast: morphometric perspectives on growth, differentiation, turnover and deposition of fibrin-type fibrinoid during gestation. *Placenta* 2001;22:628–38.
 122. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The “Great Obstetrical Syndromes” are associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;204:193–201.
 123. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 2008;359:61–73.
 124. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev* 2005;85:571–633.
 125. Warner MJ, Ozanne SE. Mechanisms involved in the developmental programming of adulthood disease. *Biochem J* 2010;427:333–47.
 126. Burton GJ, Fowden AL, Thornburg KL. Placental origins of chronic disease. *Physiol Rev* 2016;96:1509–65.
 127. Chisholm KM, Folkins AK. Placental and clinical characteristics of term small-for-gestational-age neonates: a case-control study. *Pediatr Dev Pathol* 2016;19:37–46.
 128. Gibbins KJ, Silver RM, Pinar H, Reddy UM, Parker CB, Thorsten V, et al. Stillbirth, hypertensive disorders of pregnancy, and placental pathology. *Placenta* 2016;43:61–8.
 129. Naeye RL. Pregnancy hypertension, placental evidences of low uteroplacental blood flow, and spontaneous premature delivery. *Hum Pathol* 1989;20:441–4.
 130. Redline RW. The clinical implications of placental diagnoses. *Semin Perinatol* 2015;39:2–8.
 131. Christensen RD, Lambert DK, Richards DS. Estimating the nucleated red blood cell ‘emergence time’ in neonates. *J Perinatol* 2014;34:116–9.
 132. Ferber A, Fridel Z, Weissmann-Brenner A, Minior VK, Divon MY. Are elevated fetal nucleated red blood cell counts an indirect reflection of enhanced erythropoietin activity? *Am J Obstet Gynecol* 2004;190:1473–5.
 133. Teramo KA, Widness JA. Increased fetal plasma and amniotic fluid erythropoietin concentrations: markers of intrauterine hypoxia. *Neonatology* 2009;95:105–16.
 134. Mayhew TM. Changes in fetal capillaries during preplacental hypoxia: growth, shape remodelling and villous capillarization in placentae from high-altitude pregnancies. *Placenta* 2003;24:191–8.
 135. Altshuler G. Role of the placenta in perinatal pathology (revisited). *Pediatr Pathol Lab Med* 1996;16:207–33.
 136. Andres RL, Kuyper W, Resnik R, Piacquadio KM, Benirschke K. The association of maternal floor infarction of the placenta with adverse perinatal outcome. *Am J Obstet Gynecol* 1990;163:935–8.
 137. Fox H. Pathology of the placenta. London: W B Saunders; 1978.
 138. Faye-Petersen OM, Ernst LM. Maternal floor infarction and massive perivillous fibrin deposition. *Surg Pathol Clin* 2013;6:101–14.
 139. Naeye RL. Maternal floor infarction. *Hum Pathol* 1985;16:823–8.
 140. Sebire NJ, Backos M, El Gaddal S, Goldin RD, Regan L. Placental pathology, antiphospholipid antibodies, and pregnancy outcome in recurrent miscarriage patients. *Obstet Gynecol* 2003;101:258–63.
 141. Clewell WH, Manchester DK. Recurrent maternal floor infarction: a preventable cause of fetal death. *Am J Obstet Gynecol* 1983;147:346–7.
 142. Stanek J. Acute and chronic placental membrane hypoxic lesions. *Virchows Arch* 2009;455:315–22.
 143. Cremer M, Roll S, Graf C, Weimann A, Buhner C, Dame C. Nucleated red blood cells as marker for an increased risk of unfavorable outcome and mortality in very low birth weight infants. *Early Hum Dev* 2015;91:559–63.
 144. Kil TH, Han JY, Kim JB, Ko GO, Lee YH, Kim KY, et al. A study on the measurement of the nucleated red blood cell (nRBC) count based on birth weight and its correlation with perinatal prognosis in infants with very low birth weights. *Korean J Pediatr* 2011;54:69–78.
 145. Curtin WM, Shehata BM, Khuder SA, Robinson HB, Brost BC. The feasibility of using histologic placental sections to predict newborn nucleated red blood cell counts. *Obstet Gynecol* 2002;100:305–10.
 146. Redline RW. Elevated circulating fetal nucleated red blood cells and placental pathology in term infants who develop cerebral palsy. *Hum Pathol* 2008;39:1378–84.
 147. Gersell DJ. ASCP survey on placental examination. American Society of Clinical Pathologists. *Am J Clin Pathol* 1998;109:127–43.
 148. Hermansen MC. Nucleated red blood cells in the fetus and newborn. *Arch Dis Child Fetal Neonatal Ed* 2001;84:F211–5.
 149. Maier RF, Gunther A, Vogel M, Dudenhausen JW, Obladen M. Umbilical venous erythropoietin and umbilical arterial pH in relation to morphologic placental abnormalities. *Obstet Gynecol* 1994;84:81–7.
 150. Green DW, Mimouni F. Nucleated erythrocytes in healthy infants and in infants of diabetic mothers. *J Pediatr* 1990;116:129–31.
 151. Ghosh B, Mittal S, Kumar S, Dadhwal V. Prediction of perinatal asphyxia with nucleated red blood cells in cord blood of newborns. *Int J Gynaecol Obstet* 2003;81:267–71.
 152. Thilaganathan B, Salvesen DR, Abbas A, Ireland RM, Nicolaides KH. Fetal plasma erythropoietin concentration in red blood cell-isoimmunized pregnancies. *Am J Obstet Gynecol* 1992;167:1292–7.
 153. Widness JA, Teramo KA, Clemons GK, Garcia JF, Cavalieri RL, Piasecki GJ, et al. Temporal response of immunoreactive erythropoietin to acute hypoxemia in fetal sheep. *Pediatr Res* 1986;20:15–9.
 154. Bedrick AD. Nucleated red blood cells and fetal hypoxia: a biologic marker whose ‘timing’ has come? *J Perinatol* 2014;34:85–6.
 155. Blackwell SC, Hallak M, Hotra JW, Refuerzo J, Hassan SS, Sokol RJ, et al. Timing of fetal nucleated red blood cell count elevation in response to acute hypoxia. *Biol Neonate* 2004;85:217–20.
 156. Kaufmann P, Bruns U, Leiser R, Luckhardt M, Winterhager E. The fetal vascularisation of term human placental villi. II. Intermediate and terminal villi. *Anat Embryol* 1985;173:203–14.
 157. Kaufmann P, Sen DK, Schweikhart G. Classification of human placental villi. I. Histology. *Cell Tissue Res* 1979;200:409–23.
 158. Kumazaki K, Nakayama M, Suehara N, Wada Y. Expression of vascular endothelial growth factor, placental growth factor, and their receptors Flt-1 and KDR in human placenta under pathologic conditions. *Hum Pathol* 2002;33:1069–77.

159. Stanek J. Hypoxic patterns of placental injury: a review. *Arch Pathol Lab Med* 2013;137:706–20.
160. Man J, Hutchinson JC, Heazell AE, Ashworth M, Jeffrey I, Sebire NJ. Stillbirth and intrauterine fetal death: role of routine histopathological placental findings to determine cause of death. *Ultrasound Obstet Gynecol* 2016;48:579–84.
161. Bukowski R, Carpenter M, Conway D, Coustan D, Dudley DJ, Goldenberg RL, et al. Causes of death among stillbirths. *J Am Med Assoc* 2011;306:2459–68.
162. Benirschke K, Driscoll SG. The pathology of the human placenta. New York: Springer-Verlag; 1967.
163. Bane AL, Gillan JE. Massive perivillous fibrinoid causing recurrent placental failure. *Br J Obstet Gynaecol* 2003;110:292–5.
164. Bendon RW, Hommel AB. Maternal floor infarction in autoimmune disease: two cases. *Pediatr Pathol Lab Med* 1996;16:293–7.
165. Mandsager NT, Bendon R, Mostello D, Rosenn B, Miodovnik M, Siddiqi TA. Maternal floor infarction of the placenta: prenatal diagnosis and clinical significance. *Obstet Gynecol* 1994;83(5 Pt 1):750–4.
166. Nickel RE. Maternal floor infarction: an unusual cause of intrauterine growth retardation. *Am J Dis Child* 1988;142:1270–1.
167. Redline RW, Jiang JG, Shah D. Discordancy for maternal floor infarction in dizygotic twin placentas. *Hum Pathol* 2003;34:822–4.
168. Sebire NJ, Backos M, Goldin RD, Regan L. Placental massive perivillous fibrin deposition associated with antiphospholipid antibody syndrome. *Br J Obstet Gynaecol* 2002;109:570–3.
169. Vernof KK, Benirschke K, Kephart GM, Wasmoe TL, Gleich GJ. Maternal floor infarction: relationship to X cells, major basic protein, and adverse perinatal outcome. *Am J Obstet Gynecol* 1992;167:1355–63.
170. Al-Adnani M, Kiho L, Scheimberg I. Recurrent placental massive perivillous fibrin deposition associated with polymyositis: a case report and review of the literature. *Pediatr Dev Pathol* 2008;11:226–9.
171. Hung NA, Jackson C, Nicholson M, Highton J. Pregnancy-related polymyositis and massive perivillous fibrin deposition in the placenta: are they pathogenetically related? *Arthritis Rheum* 2006;55:154–6.
172. Frank HG, Malekzadeh F, Kertschanska S, Crescimanno C, Castellucci M, Lang I, et al. Immunohistochemistry of two different types of placental fibrinoid. *Acta Anat (Basel)* 1994;150:55–68.
173. Sun CC, Revell VO, Belli AJ, Viscardi RM. Discrepancy in pathologic diagnosis of placental lesions. *Arch Pathol Lab Med* 2002;126:706–9.
174. McDonald B, Moore L. IUGR and laminar necrosis of the placental membranes. *Pediatr Dev Pathol* 2006;9:170.
175. Kidron D, Bernheim J, Aviram R. Placental findings contributing to fetal death, a study of 120 stillbirths between 23 and 40 weeks gestation. *Placenta* 2009;30:700–4.
176. Amir H, Weintraub A, Aricha-Tamir B, Apel-Sarid L, Holcberg G, Sheiner E. A piece in the puzzle of intrauterine fetal death: pathological findings in placentas from term and preterm intrauterine fetal death pregnancies. *J Matern Fetal Neonatal Med* 2009;22:759–64.
177. Rayburn W, Sander C, Barr Jr M, Rygiel R. The stillborn fetus: placental histologic examination in determining a cause. *Obstet Gynecol* 1985;65:637–41.
178. Genest DR. Estimating the time of death in stillborn fetuses: II. Histologic evaluation of the placenta; a study of 71 stillborns. *Obstet Gynecol* 1992;80:585–92.
179. Stanek J, Biesiada J. Relation of placental diagnosis in stillbirth to fetal maceration and gestational age at delivery. *J Perinat Med* 2014;42:457–71.
180. Beeksmas FA, Erwich JJ, Khong TY. Placental fetal vascular thrombosis lesions and maternal thrombophilia. *Pathology* 2012;44:24–8.
181. Parast MM, Crum CP, Boyd TK. Placental histologic criteria for umbilical blood flow restriction in unexplained stillbirth. *Hum Pathol* 2008;39:948–53.
182. Boyd TK. The placenta in intrauterine demise. *APMIS* 2018;126:621–5.
183. Langley FA. The perinatal postmortem examination. *J Clin Pathol* 1971;24:159–69.
184. Bain A. The perinatal autopsy. Oxford: Blackwell Scientific Publications; 1974.
185. Genest DR, Williams MA, Greene MF. Estimating the time of death in stillborn fetuses: I. Histologic evaluation of fetal organs; an autopsy study of 150 stillborns. *Obstet Gynecol* 1992;80:575–84.
186. Redline RW. Clinical and pathological umbilical cord abnormalities in fetal thrombotic vasculopathy. *Hum Pathol* 2004;35:1494–8.
187. Garrod A, Batra G, Ptacek I, Heazell AE. Duration and method of tissue storage alters placental morphology – implications for clinical and research practice. *Placenta* 2013;34:1116–9.
188. Kraus FT, Acheen VI. Fetal thrombotic vasculopathy in the placenta: cerebral thrombi and infarcts, coagulopathies, and cerebral palsy. *Hum Pathol* 1999;30:759–69.
189. Korteweg FJ, Gordijn SJ, Timmer A, Holm JP, Ravise JM, Erwich JJ. A placental cause of intra-uterine fetal death depends on the perinatal mortality classification system used. *Placenta* 2008;29:71–80.
190. Bustamante Helfrich B, Chilukuri N, He H, Cerda SR, Hong X, Wang G, et al. Maternal vascular malperfusion of the placental bed associated with hypertensive disorders in the Boston Birth Cohort. *Placenta* 2017;52:106–13.
191. Maloney KF, Heller D, Baergen RN. Types of maternal hypertensive disease and their association with pathologic lesions and clinical factors. *Fetal Pediatr Pathol* 2012;31:319–23.
192. Weiner E, Feldstein O, Tamayev L, Grinstein E, Barber E, Bar J, et al. Placental histopathological lesions in correlation with neonatal outcome in preeclampsia with and without severe features. *Pregnancy Hypertens* 2018;12:6–10.
193. Parks WT. Manifestations of hypoxia in the second and third trimester placenta. *Birth Defects Res* 2017;109:1345–57.
194. Chang KT, Keating S, Costa S, Machin G, Kingdom J, Shannon P. Third-trimester stillbirths: correlative neuropathology and placental pathology. *Pediatr Dev Pathol* 2011;14:345–52.
195. Lannaman K, Romero R, Chaiworapongsa T, Kim YM, Korzeniewski SJ, Maymon E, et al. Fetal death: an extreme manifestation of maternal anti-fetal rejection. *J Perinat Med* 2017;45:851–68.
196. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004;5:266–71.

197. Betz AG. Immunology: tolerating pregnancy. *Nature* 2012;490:47–8.
198. Bonney EA. Immune regulation in pregnancy: a matter of perspective? *Obstet Gynecol Clin North Am* 2016;43:679–98.
199. Erlebacher A. Why isn't the fetus rejected? *Curr Opin Immunol* 2001;13:590–3.
200. Erlebacher A. Immunology of the maternal-fetal interface. *Annu Rev Immunol* 2013;31:387–411.
201. Hemberger M. Immune balance at the foeto-maternal interface as the fulcrum of reproductive success. *J Reprod Immunol* 2013;97:36–42.
202. Munoz-Suano A, Hamilton AB, Betz AG. Gimme shelter: the immune system during pregnancy. *Immunol Rev* 2011;241:20–38.
203. PrabhuDas M, Bonney E, Caron K, Dey S, Erlebacher A, Fazleabas A, et al. Immune mechanisms at the maternal-fetal interface: perspectives and challenges. *Nat Immunol* 2015;16:328–34.
204. Rowe JH, Ertelt JM, Xin L, Way SS. Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. *Nature* 2012;490:102–6.
205. Lee J, Romero R, Chaiworapongsa T, Dong Z, Tarca AL, Xu Y, et al. Characterization of the fetal blood transcriptome and proteome in maternal anti-fetal rejection: evidence of a distinct and novel type of human fetal systemic inflammatory response. *Am J Reprod Immunol* 2013;70:265–84.
206. Lee J, Romero R, Xu Y, Kim JS, Park JY, Kusanovic JP, et al. Maternal HLA panel-reactive antibodies in early gestation positively correlate with chronic chorioamnionitis: evidence in support of the chronic nature of maternal anti-fetal rejection. *Am J Reprod Immunol* 2011;66:510–26.
207. Bang H, Bae GE, Park HY, Kim YM, Choi SJ, Oh SY, et al. Chronic placental inflammation in twin pregnancies. *J Pathol Transl Med* 2015;49:489–96.
208. Redline RW, Patterson P. Villitis of unknown etiology is associated with major infiltration of fetal tissue by maternal inflammatory cells. *Am J Pathol* 1993;143:473–9.
209. Romero R, Whitten A, Korzeniewski SJ, Than NG, Chaemsaitong P, Miranda J, et al. Maternal floor infarction/massive perivillous fibrin deposition: a manifestation of maternal antifetal rejection? *Am J Reprod Immunol* 2013;70:285–98.
210. Redline RW. Villitis of unknown etiology: noninfectious chronic villitis in the placenta. *Hum Pathol* 2007;38:1439–46.
211. Derricott H, Jones RL, Heazell AE. Investigating the association of villitis of unknown etiology with stillbirth and fetal growth restriction – a systematic review. *Placenta* 2013;34:856–62.
212. Man J, Hutchinson JC, Ashworth M, Jeffrey I, Heazell AE, Sebire NJ. Organ weights and ratios for postmortem identification of fetal growth restriction: utility and confounding factors. *Ultrasound Obstet Gynecol* 2016;48:585–90.
213. Bonetti LR, Ferrari P, Trani N, Maccio L, Laura S, Giuliana S, et al. The role of fetal autopsy and placental examination in the causes of fetal death: a retrospective study of 132 cases of stillbirths. *Arch Gynecol Obstet* 2011;283:231–41.
214. Hayati AR, Khong TY, Zainul R. The usefulness of limited placental sampling in stillbirths. *Malays J Pathol* 1998;20:99–102.
215. Page JM, Bardsley T, Thorsten V, Allshouse AA, Varner MW, Debbink MP, et al. Stillbirth associated with infection in a diverse U.S. Cohort. *Obstet Gynecol* 2019;134:1187–96.
216. Romero R, Miranda J, Chaemsaitong P, Chaiworapongsa T, Kusanovic JP, Dong Z, et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. *J Matern Fetal Neonatal Med* 2015;28:1394–409.
217. Romero R, Miranda J, Chaiworapongsa T, Chaemsaitong P, Gotsch F, Dong Z, et al. A novel molecular microbiologic technique for the rapid diagnosis of microbial invasion of the amniotic cavity and intra-amniotic infection in preterm labor with intact membranes. *Am J Reprod Immunol* 2014;71:330–58.
218. Romero R, Miranda J, Chaiworapongsa T, Chaemsaitong P, Gotsch F, Dong Z, et al. Sterile intra-amniotic inflammation in asymptomatic patients with a sonographic short cervix: prevalence and clinical significance. *J Matern Fetal Neonatal Med* 2015;28:1343–59.
219. Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaitong P, Gotsch F, et al. Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. *Am J Reprod Immunol* 2014;72:458–74.
220. Romero R, Miranda J, Kusanovic JP, Chaiworapongsa T, Chaemsaitong P, Martinez A, et al. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. *J Perinat Med* 2015;43:19–36.
221. Kovo M, Schreiber L, Ben-Haroush A, Asalee L, Seadia S, Golan A, et al. The placental factor in spontaneous preterm labor with and without premature rupture of membranes. *J Perinat Med* 2011;39:423–9.
222. Hsu CD, Meaddough E, Aversa K, Hong SF, Lee IS, Bahodo-Singh RO, et al. Dual roles of amniotic fluid nitric oxide and prostaglandin E2 in preterm labor with intra-amniotic infection. *Am J Perinatol* 1998;15:683–7.
223. Maddipati KR, Romero R, Chaiworapongsa T, Chaemsaitong P, Zhou SL, Xu Z, et al. Lipidomic analysis of patients with microbial invasion of the amniotic cavity reveals up-regulation of leukotriene B4. *FASEB J* 2016;30:3296–307.
224. Maddipati KR, Romero R, Chaiworapongsa T, Zhou SL, Xu Z, Tarca AL, et al. Eicosanomic profiling reveals dominance of the epoxygenase pathway in human amniotic fluid at term in spontaneous labor. *FASEB J* 2014;28:4835–46.
225. Park JY, Romero R, Lee J, Chaemsaitong P, Chaayasit N, Yoon BH. An elevated amniotic fluid prostaglandin F2alpha concentration is associated with intra-amniotic inflammation/infection, and clinical and histologic chorioamnionitis, as well as impending preterm delivery in patients with preterm labor and intact membranes. *J Matern Fetal Neonatal Med* 2016;29:2563–72.
226. Romero R, Emamian M, Quintero R, Wan M, Hobbins JC, Mitchell MD. Amniotic fluid prostaglandin levels and intra-amniotic infections. *Lancet (London, England)* 1986;1:1380.
227. Romero R, Emamian M, Wan M, Quintero R, Hobbins JC, Mitchell MD. Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor. *Am J Obstet Gynecol* 1987;157:1461–7.
228. Romero R, Wu YK, Mazor M, Hobbins JC, Mitchell MD. Amniotic fluid prostaglandin E2 in preterm labor. *Prostaglandins Leukot Essent Fatty Acids* 1988;34:141–5.
229. Romero R, Wu YK, Mazor M, Oyarzun E, Hobbins JC, Mitchell MD. Amniotic fluid arachidonate lipoxygenase metabolites in preterm labor. *Prostaglandins Leukot Essent Fatty Acids* 1989;36:69–75.
230. Romero R, Wu YK, Sirtori M, Oyarzun E, Mazor M, Hobbins JC, et al. Amniotic fluid concentrations of prostaglandin F2 alpha,

- 13,14-dihydro-15-keto-prostaglandin F2 alpha (PGFM) and 11-deoxy-13,14-dihydro-15-keto-11, 16-cyclo-prostaglandin E2 (PGEM-LL) in preterm labor. *Prostaglandins* 1989;37:149–61.
231. Lim SY, Kim YH, Kim CH, Cho MK, Kim JW, Kang WD, et al. The effect of a Foley catheter balloon on cervical ripening. *J Obstet Gynaecol* 2013;33:830–8.
232. Page JM, Christiansen-Lindquist L, Thorsten V, Parker CB, Reddy UM, Dudley DJ, et al. Diagnostic tests for evaluation of stillbirth: results from the stillbirth collaborative research network. *Obstet Gynecol* 2017;129:699–706.
233. Jones F, Thibon P, Guyot M, Molin A, Jeanne-Pasquier C, Guillois B, et al. Practice of pathological examinations in stillbirths: a 10-year retrospective study. *J Gynecol Obstet Hum Reprod* 2017;46:61–7.
234. Walsh CA, Vallerie AM, Baxi LV. Etiology of stillbirth at term: a 10-year cohort study. *J Matern Fetal Neonatal Med* 2008;21:493–501.
235. Horn LC, Langner A, Stiehl P, Wittekind C, Faber R. Identification of the causes of intrauterine death during 310 consecutive autopsies. *Eur J Obstet Gynecol Reprod Biol* 2004;113:134–8.
236. Ernst LM, Minturn L, Huang MH, Curry E, Su EJ. Gross patterns of umbilical cord coiling: correlations with placental histology and stillbirth. *Placenta* 2013;34:583–8.
237. Folgosa E, Gonzalez C, Osman NB, Hagerstrand I, Bergstrom S, Ljungh A. A case control study of chorioamniotic infection and histological chorioamnionitis in stillbirth. *APMIS* 1997;105:329–36.
238. Moyo SR, Hagerstrand I, Nystrom L, Tswana SA, Blomberg J, Bergstrom S, et al. Stillbirths and intrauterine infection, histologic chorioamnionitis and microbiological findings. *Int J Gynaecol Obstet* 1996;54:115–23.
239. Tantbirojn P, Saleemuddin A, Sirois K, Crum CP, Boyd TK, Tworoger S, et al. Gross abnormalities of the umbilical cord: related placental histology and clinical significance. *Placenta* 2009;30:1083–8.
240. Altemani AM. Thrombosis of fetal placental vessels. A quantitative study in placentas of stillbirths. *Pathology, research and practice* 1987;182:685–9.
241. Lahra MM, Gordon A, Jeffery HE. Chorioamnionitis and fetal response in stillbirth. *Am J Obstet Gynecol* 2007;196:229.e1–4.
242. Feeley L, Mooney EE. Villitis of unknown aetiology: correlation of recurrence with clinical outcome. *J Obstet Gynaecol* 2010;30:476–9.
243. Menghrajani P, Osterheld MC. Significance of hemorrhagic endovasculitis in placentae from stillbirths. *Pathol Res Pract* 2008;204:389–94.
244. Sander CM, Gilliland D, Flynn MA, Swart-Hills LA. Risk factors for recurrence of hemorrhagic endovasculitis of the placenta. *Obstet Gynecol* 1997;89:569–76.
245. Sander CM, Gilliland D, Richardson A, Foley KM, Fredericks J. Stillbirths with placental hemorrhagic endovasculitis: a morphologic assessment with clinical implications. *Arch Pathol Lab Med* 2005;129:632–8.
246. Stevens NG, Sander CH. Placental hemorrhagic endovasculitis: risk factors and impact on pregnancy outcome. *Int J Gynaecol Obstet* 1984;22:393–7.

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