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Disorders of placental villous maturation are present in one third of cases with spontaneous preterm labor

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Abstract

Background: Spontaneous preterm labor is an obstetrical syndrome accounting for approximately 65-70% of preterm births, the latter being the most frequent cause of neonatal death and the second most frequent cause of death in children less than five years of age worldwide.

Objectives: The purpose of this study was to determine and compare to uncomplicated pregnancies 1) the frequency of placental disorders of villous maturation in spontaneous preterm labor; 2) the frequency of other placental morphologic characteristics associated with the preterm labor syndrome; and 3) the distribution of these lesions according to gestational age at delivery and their severity.

Methods: A case-control study of singleton pregnant women was conducted that included 1) uncomplicated pregnancies (controls, n=944) and 2) pregnancies with spontaneous preterm labor (cases, n= 438). All placentas underwent histopathologic examination. Patients with chronic maternal diseases (e.g., chronic hypertension, diabetes mellitus, renal disease, thyroid disease, asthma, autoimmune disease, and coagulopathies), fetal malformations, chromosomal abnormalities, multifetal gestation, preeclampsia, eclampsia, preterm prelabor rupture of the fetal membranes, gestational hypertension, gestational diabetes mellitus, and hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome were excluded from the study.

Results: Compared to controls, the most prevalent placental lesions were the disorders of villous maturation (31.8% [106/333] including delayed villous maturation 18.6% [62/333] vs. 1.4% [6/442], $q<0.0001$, prevalence ratio 13.7; and accelerated villous maturation 13.2% [44/333] vs. 0% [0/442], $q<0.001$). Other lesions in decreasing order of prevalence included hypercapillarized villi (15.6% [68/435] vs. 3.5% [33/938], $q<0.001$, prevalence ratio 4.4); nucleated red blood cells (1.1% [5/437] vs. 0% [0/938], $q<0.01$); chronic inflammatory lesions (47.9% [210/438] vs. 29.9% [282/944], $q<0.0001$, prevalence ratio 1.6); fetal inflammatory response (30.1% [132/438] vs. 23.2% [219/944], $q<0.05$, prevalence ratio 1.3); maternal inflammatory response (45.5% [195/438] vs. 36.1% [341/944], $q<0.01$, prevalence ratio 1.2); and maternal vascular malperfusion (44.5% [195/438] vs. 35.7% [337/944], $q<0.01$, prevalence ratio 1.2). Accelerated villous maturation did not show gestational age-dependent association with any other placental lesion while delayed villous maturation showed a gestational age-dependent association with acute placental inflammation (q-value= 0.005).

Conclusions: Disorders of villous maturation are present in nearly one-third of the cases of spontaneous preterm labor.

Keywords

accelerated villous maturation; acute placental inflammation; chronic placental inflammation; delayed villous maturation; hypercapillarized villi; maternal anti-fetal rejection; maternal-fetal

inflammatory response; maternal vascular malperfusion; nucleated red blood cells; placental pathology

Introduction

Preterm birth affected nearly 11% of livebirths globally in 2014 and was the prime cause of death in children under five years of age, accounting for 35% of deaths among neonates [1, 2]. It is the leading cause of perinatal mortality and morbidity worldwide [1, 3-8]. In addition, the surviving infants have higher rates of long-term morbidity, including neurologic and developmental disabilities, compared to infants born at full term [9]. Among all singleton preterm births, spontaneous preterm birth accounts for 65-70% of all preterm births, while 30-35% can be attributed to indicated preterm delivery [10, 11]. In order to develop effective preventive measures to reduce the incidence of spontaneous preterm labor, there is a need to understand the cause of this great obstetrical syndrome [1, 12].

The hallmark of a normal third-trimester (>32 weeks of gestation) placenta is the approximation of syncytiotrophoblasts with a villous capillary endothelium and the formation of vasculo-syncytial membranes [13-16]. These vasculo-syncytial membranes of the terminal villi are the principal sites of gaseous exchange in the placenta [15, 17, 18]. A reduction in the number of vasculo-syncytial membranes suggests either villous immaturity or villous regressive changes [19], and it has been suggested that the fetuses whose placentas show a low vasculo-syncytial membrane count experience a high incidence of hypoxic complications [19]. Defective maturation of the villous tree can cause diminution of vasculo-syncytial membranes resulting in placental dysfunction and fetal hypoxia [20-25].

We hypothesized that abnormalities of the placental vasculature impair the nutritional or exchange function of the placenta. Moreover, given that abnormal development or maturation of the placental villous tree and its vasculature can display qualitative and quantitative deviations from normal villous development [25, 26] and may also affect placental exchange by decreasing the mass of terminal villi available for exchange [27], we examined the maturation of placental villi in cases of spontaneous preterm delivery.

The objectives of this study were to determine the following: 1) frequency of placental disorders of villous maturation in spontaneous preterm labor; 2) frequency of other placental morphologic characteristics associated with the preterm labor syndrome; and 3) distribution of these lesions according to gestational age at delivery. The data were compared to findings of lesions observed in placentas from uncomplicated pregnancies.

MATERIALS AND METHODS

This was a retrospective observational study of spontaneous preterm labor that occurred among pregnant women recruited into cohort studies between January 2004 and January 2016 at Hutzel Women's Hospital of the Detroit Medical Center, Detroit, Michigan, 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 pregnant women with spontaneous preterm labor and intact membranes (n=438) who gave birth prior to 37 weeks of gestation [1]. The control group (n=944) comprised placentas between 37-42 weeks of gestation.

Inclusion criteria for term control placentas were pregnant women who had no medical or obstetrical complications and delivered a singleton, term neonate with a 5-minute Apgar score ≥7 and birthweight between the 10th and 90th percentiles, according to a U.S reference population [28]. Excluded from the study were pregnant women who presented clinical symptoms of 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, spontaneous preterm labor, and HELLP syndrome (Hemolysis, Elevated Liver enzymes, and Low Platelet count).

Placental histopathologic study

Placentas were sampled using systematic random and targeting sampling techniques as previously described [29]. Slides from the cases and controls, were first stained with hematoxylin and eosin and then were reviewed by placental pathologists blinded to the pregnancy outcome and all previous histopathologic diagnoses. Placental lesions were described according to the nomenclature recommended by the Amsterdam Placental Workshop Group [30], except for disorders of villous maturation [25, 26, 31-33].

Immunohistochemistry marker CD15 was also utilized whereby the “immature” CD15-positive endothelium was considered as a diagnostic marker of delayed villous maturity and chronic placental dysfunction [34, 35]. The expression of CD15 in the macrovasculature (chorionic plate and stem vessels) and microvasculature (terminal villi) whereby ≥50% of villi exhibit positivity was considered as pathological placental villous immaturity [34, 35]. For placentas of spontaneous preterm labor (cases, n=333) as well as placentas from pregnancies with normal outcome (controls, n=405), two blocks each were stained with CD-15 according to the technique described by Seidmann et al [34, 35]. Blood vessels of the chorionic plate, stem villi, and chorionic villi were counted, and the ratio was calculated as positively stained endothelial cells over the total number of vessels counted [34, 35]. Only the blood vessels that showed circumferential staining reaction (implying all the endothelial cells in a blood vessel) were considered positive. Sections were first scanned using a 4X objective. Moreover, only those cases for which the chorionic plate, stem villous, and chorionic villous vessels showed a 50% or more positive CD-15 endothelial reaction were considered positive. The ratio was calculated as CD-15 positively stained endothelial cells over the total number of vessels (500 vessels) counted in one section. It is noteworthy that many sections did not show chorionic plate vessels. However, if the chorionic villi showed a 50% CD-15 positive reaction in these sections, they were then evaluated for delayed villous maturation.

Additionally, the immunohistochemical marker CD-31 for vascular endothelia was utilized [36]. CD-31 served as a quality control marker that was consistently expressed by all placental vessels throughout the entire duration of pregnancy but was not affected by villous maturation abnormalities [34, 35, 37]. CD-31 also highlighted hypercapillarized villi that lacked vasculo-syncytial membranes and showed centrally placed blood vessels.

Moreover, nucleated red blood cells were counted using a high-power objective (40X), whereby 10 fields were counted for the presence of nucleated red blood cells. A count was considered abnormal when >10 nucleated red blood cells in a 10 high-power field were observed. It should be noted that a value of >10 nucleated red blood cells per a 10 high-power field corroborates with an absolute value of $2.5 \times 10^3/mm^3$ or more and that it can be considered as an elevated nucleated red cell count with high sensitivity and specificity [38].

Placental histopathologic definitions are displayed in Supplementary Table 1. A schematic representation of a normal villous tree and the disorders of villous maturation is depicted in Figure 1.

Statistical Analysis

Analysis of clinical characteristics and gross morphology: Continuous data were compared between groups with the Welch's t-test or an analysis of variance [39]. For categorical variables, proportions were compared between groups with the Fisher's exact test [40]. A nominal p-value <0.05 was considered statistically significant.

Analysis of placental pathology: We calculated the prevalence of a lesion occurring in a study group (e.g., preterm labor) as follows: the proportion of placentas with the lesion in that group and the prevalence ratio between two groups as the ratio of prevalence of the lesion in the two groups [41]. The nominal p-values from the exact tests were adjusted for multiple comparisons with the Benjamini-Hochberg procedure [42]. An adjusted p-value (q-value) < 0.05 was considered statistically significant.

All analyses were performed in the R statistical software [43].

RESULTS

Demographic and clinical characteristics of the study population

This placental study comprised 438 cases and 944 controls. The demographic characteristics of the study groups are presented in Table 1. Cases had lower median maternal height and pre-pregnancy body mass index (p<0.01 for both), lower maternal weight (p=0.02), African-American ethnicity (p=0.01), and, higher tobacco use (p<0.0001), alcohol abuse (p<0.0001), drug abuse in pregnancy (p<0.0001), history of previous preterm delivery (p<0.0001), spontaneous labor (p<0.0001), composite neonatal morbidity (p<0.0001), and early neonatal death (p<0.0001) than the controls. Women with preterm labor had lower rates of induction of labor (p<0.0001), augmented labor (p<0.0001), and cesarean delivery (p=0.001). In addition, cases had lower median gestational age at delivery (p<0.0001),

birthweight (p<0.0001), and ponderal index (p<0.0001). There was no difference in the frequency of male neonates and a neonatal ponderal index < 10th percentile.

Gross morphologic findings

Comparisons of the parameters of placental size and gross morphological features are displayed in Table 2.

Placental microscopic histopathologic findings

The frequency of placental lesions is shown in Table 3 and Table 4. Disorders of villous maturation (31.8% [106/333]) were more prevalent in cases compared to controls: delayed villous maturation (cases 18.6% [62/333] vs. controls 1.4% [6/442], q <0.0001, prevalence ratio 13.7) and accelerated villous maturation (cases 13.2% [44/333] vs. controls 0% [0/442], q <0.001) (Figure 2).

Other lesions significantly more prevalent in cases compared to controls included hypercapillarized villi (cases 15.6% [68/435] vs. controls 3.5% [33/938], q <0.001, prevalence ratio 4.4); nucleated red blood cells (cases 1.1% [5/437] vs. controls 0% [0/938], q <0.01); chronic inflammatory lesions (cases 47.9% [210/438] vs. controls 29.9% [282/944], q<0.0001, prevalence ratio 1.6); fetal inflammatory response (cases 30.1% [132/438] vs. controls 23.2% [219/944], q<0.05, prevalence ratio 1.3); maternal inflammatory response (cases 45.5% [195/438] vs. 36.1% [341/944], q<0.01, prevalence ratio 1.2); and maternal vascular malperfusion (cases 44.5% [195/438] vs. 35.7% [337/944], q<0.01, prevalence ratio 1.2) (Table 3).

Acute placental inflammatory lesions—The frequency of the maternal inflammatory response and the fetal inflammatory response was significantly higher, respectively (maternal inflammatory response: cases 45.5% [195/438] vs. controls 36.1% [341/944], q<0.01; fetal inflammatory response: cases 30.1% [132/438] vs. controls 23.2% [219/944], q<0.05) (Table 3). The frequency of acute placental inflammatory lesions increased as the gestational age at delivery decreased (Table 4; Figure 3).

Chronic placental inflammatory lesions—The frequency of chronic inflammatory lesions was higher in cases (47.9% [210/438]) than in controls (29.9% [282/944], q<0.0001) (Table 3). The frequency of chronic deciduitis, particularly the lymphoplasmacytic type, was significantly higher in cases than in controls (q<0.0001). The rate of chronic chorioamnionitis at grade 1/stage 1 (q<0.0001), grade 1/stage 2 (q=0.02), and grade 2/stage 2 (q<0.01) was higher in cases than in controls. The rate of severe chronic inflammatory lesions was significantly higher in cases than in controls (q=0.01) (Figure 3). There was no difference between cases and controls in the frequency of villitis of unknown etiology with high-grade lesions, chronic chorioamnionitis grade 2/stage 1, chronic histiocytic intervillositis, villitis of infectious origin, and eosinophilic T-cell vasculitis.

Maternal vascular malperfusion—The frequency of lesions of maternal vascular malperfusion was higher in cases than in controls (cases, 44.5% [195/438] vs. controls, 35.7% [337/944], q<0.01) (Table 3). The frequency of maternal vascular malperfusion,

particularly villous changes (q<0.05) and vascular lesions (q<0.0001), was significantly higher in cases than in controls (q=0.0004). Among villous changes, the rates of increased intervillous fibrin deposition (q<0.01) and distal villous hypoplasia (q<0.01) were higher in cases than controls. There was no difference in the rate of villous infarct(s), increased syncytial knots, and villous agglutination between cases and controls. Among vascular lesions, the rates of persistent muscularization of basal plate arteries (q<0.01), mural hypertrophy of decidual arterioles (q=0.01), and ≥ 2 lesions of maternal vascular malperfusion (q<0.01) were highest with prevalence ratios of 1.4, 2.5, and 1.8, respectively.

Fetal vascular malperfusion—There was no difference in the rate of fetal vascular malperfusion between cases and controls (Table 3).

Hypoxic histologic patterns of placental injury—The frequency of hypercapillarized villi and nucleated red blood cells was higher in cases than in controls (hypercapillarized villi: cases, 15.6% [68/435] vs. controls, 3.5% [33/938], q<0.001; nucleated red blood cells: cases, 1.1% [5/437] vs. controls, 0% [0/938], q<0.01) (Table 3). There was no difference in the frequency of massive perivillous fibrin deposition and laminar necrosis of the decidua capsularis between cases and controls.

Placental histological lesions according to gestational age at delivery—Seventy-three percent (321/438) of the study participants delivered between 34 and 36.9 weeks of gestation, 12% (53/438) delivered between 28 and 33 weeks of gestation, 8% (34/438) delivered between 24 and 27 weeks of gestation, and 7% (30/438) delivered < 24 weeks of gestation. The frequency of placental histological lesions according to the gestational-age period at delivery is shown in Table 4.

The frequency of acute placental inflammatory lesions was higher (84.3% [54/64]) when delivery occurred < 28 weeks of gestation compared to 39.57% (148/374) when delivery occurred > 28 weeks of gestation. The frequency of maternal and fetal inflammatory responses for preterm delivery < 28 weeks of gestation was also higher: 84.3% (54/64) and 46.9% (30/64), respectively, relative to 37.7% (141/374) and 27.27% (102/374) respectively, when delivery occurred > 28 weeks of gestation. Alternatively, the frequency of chronic placental lesions for a gestational age > 28 weeks was higher than those < 28 weeks: 49.1% (184/374) and 40.6% (26/64), respectively.

The frequency of lesions of maternal vascular malperfusion was higher at a gestational age < 28 weeks (53.1% [34/64] than > 28 weeks (43% [161/374]), and both villous and vascular lesions were higher in those born < 28 weeks (21.87% [14/64])) and 42.18 % [27/64]) than those born > 28 weeks (20% [75/374] and 28.6% [107/374], respectively (Figure 3). The highest frequency of lesions of maternal vascular malperfusion was 55.9% (19/34) in cases between 24 and 27 weeks of gestation, and the highest frequency of ≥ 2 lesions of maternal vascular malperfusion (26.7% [8/30]) occurred in cases < 24 weeks of gestation. There was no difference in the frequency of placental lesions of fetal vascular malperfusion according to the different periods of gestational age.

Figure 3 displays the prevalence of the placental histological lesions according to the gestational age at delivery and the presence of labor. The rate of acute placental lesions decreased with gestational age until 36-37 weeks and then it increased. The frequency of chronic placental lesions was the highest between 30 and 33 weeks of gestation and decreased gradually during early and late gestation, depicting an inverted “U” shaped distribution. In addition, the frequency of lesions of maternal vascular malperfusion was higher at lower gestational ages and continued to decrease as the duration of pregnancy advanced.

DISCUSSION

Principal findings of the study

Placentas of patients with spontaneous preterm labor presented the following: 1) a significantly higher prevalence of disorders of villous maturation: hypercapillarized villi, nucleated red blood cells, lesions of maternal vascular malperfusion, chronic inflammatory response, fetal inflammatory response, and maternal inflammatory response; 2) acute placental inflammatory lesions and lesions of maternal vascular malperfusion with a negative correlation to gestational age at delivery; and 3) chronic placental inflammatory lesions with an inverse U-shaped pattern, displaying the highest frequency between 28 and 32 weeks of gestation.

Results in the context of what is known

Accelerated villous maturation—Defective remodeling of the spiral arteries is the proposed pathogenesis of accelerated villous maturation [24, 44, 45], leading to malperfusion of the placenta, and thereby causing loss of function through oxidative and endoplasmic reticulum stress, decreased surface area, and damage to the syncytiotrophoblast [46]. It has been hypothesized that inadequate physiological transformation of the uterine arteries is the principal mechanism by which oxidative stress may be induced in the placenta [17]. The suggestion is that the spiral arteries would remain vasoreactive by retaining their muscular coats, which, in turn, could cause 1) episodes of vasoconstriction-vasodilatation and 2) fluctuation of partial oxygen pressure of blood in the intervillous space [17, 47]. It has also been proposed that accelerated villous maturation may be either a compensatory mechanism to increase nutrient transfer surface area or a histologic marker of oxidative damage [48-52]. Consequently, there is an altered development of the fetal organs as well as the increased risk of fetal growth restriction, preterm delivery, spontaneous preterm labor, and brain injury [53-60]. These propositions notwithstanding, the extent to which a reduction of the spiral arteries stimulates terminal villi development directly from stem villi by elongated non-branching angiogenesis remains unclear [25]. In addition, it has been reported that accelerated villous maturation may also show diminished vasculo-syncytial membranes [25, 61]. This observation implies that these lesions render the fetus hypoxic by failing to deliver oxygen despite the differences in the pathogenesis of delayed and accelerated villous maturation.

Diagnosis of accelerated villous maturation—In the current study, we found that accelerated villous maturation was present in only 13.2% (44/333) of cases with

spontaneous preterm birth. This frequency is lower than those reported in two previous studies that described the presence of accelerated villous maturation in spontaneous preterm births [48, 62]; Morgan et al [48] found accelerated villous maturation in spontaneous preterm birth to be present in 84% (26/31) of cases while Nijman et al [62] reported it in 33% (40/121) of the cases of spontaneous preterm birth, respectively. The difference in the frequency of accelerated villous maturation between these studies reflects the subjectivity in the application of diagnostic criteria [63] and the lack of replicability of the diagnosis of accelerated villous maturation [45, 64-66]. Moreover, the distinction between severe accelerated villous maturation and distal villous hypoplasia, both associated with maternal vascular malperfusion, lacks consensus and requires further research and clarification [25, 30, 45, 52, 65]. Additionally, these may be focal or diffuse lesions of development and there may be ramifications with qualitative, quantitative, and/or chronological deviations from normal villous maturation [24]. In addition to the subjectivity, other factors may also affect the diagnosis of these lesions, e.g., accuracy in the assessment of gestational age, expertise on the normal villous maturation process [24], and knowledge regarding methodological factors that affect the quality of placental sampling [67]. It is worth mentioning that placental evaluation of disorders of villous maturation by those blinded to gestational age is prone to observer bias. Moreover, lack of careful attention given to sample collection, such as implementation of global or random sampling to generate data representative of the whole organ, processing, and storage, all of which are critical for reducing the number of confounding variables, can adversely influence data derived from the human placenta [29, 67-69]. Until a diagnostic immunohistochemical marker to identify hypermature villous fetal vessels is discovered to render objectivity to a morphologic interpretation of accelerated villous maturation, the interpretative quagmire of this lesion will persist.

Delayed villous maturation—Diminution of vasculo-syncytial membranes, reduction in vascularization of the chorionic villi, and insufficient development of the terminal villi remain the hallmarks of delayed villous maturation [21, 23-26, 31]. 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 spontaneous preterm labor [20, 21, 70, 71]. Prevalence of delayed villous maturation in stillbirths is accentuated, exhibiting findings consistent with fetal hypoxia [20, 71, 72]. We have recently demonstrated that the frequency of delayed villous maturation in fetal death cases was significantly increased (prevalence ratio, 21.9) as compared to controls [73]. Moreover, fetal death cases demonstrated a high prevalence of histological patterns suggestive of hypoxia, including nucleated red blood cells, hypercapillarized villi, and intravillous hemorrhage [73]. Delayed villous maturation is also associated with poor neurologic outcomes due to hypoxia [74, 75]. It has been reported that the recurrence risk of delayed villous maturation in subsequent pregnancies is approximately 5% [20].

Hypoxia of the fetus can also result from primary lesions of the villi that decrease diffusion capacity of the vasculo-syncytial membranes, rather than being caused by an impaired maternal blood flow [76, 77]. In an image-based modeling of blood flow and oxygen transfer in placental capillaries [78], it has been shown that a localized villous capillary dilation of optimal shape was found to increase oxygen transfer by up to 15% [78]. Dilated

capillaries are thought to occupy more than 35% of the total villous volume at term [23] and therefore could provide a significant enhancement to fetal oxygen uptake. The model supports the hypothesis that localized capillary dilations develop toward term to enhance oxygen transfer in the placenta without radical placental growth or remodeling [78]. In the second half of gestation, fetal growth exceeds that of placental growth [79] while the mean trophoblast thickness decreases until term [80].

Moreover, it has been reported that placentas at high altitude show increased vascularization of terminal villi [27], which could lead to an increase in the total number of capillary dilations. Enhanced oxygen transfer [81, 82] is an explanation for the increased dilation of fetal capillaries in pregnancies at high altitude; at 4300 m, maternal arterial partial pressure of oxygen is reduced by almost 50% compared to sea level [83].

The results of the image-based model [78] could also explain the hypoxia-related outcomes of delayed villous maturation [84] in addition to the raised erythropoietin levels observed in the amniotic fluid of delayed villous maturation cases [85]. The diminution and/or lack of vasculo-syncytial membranes observed in cases of delayed villous maturation could represent a lack of localized villous capillary dilations, resulting in an ineptness of the fetus to extract a requisite amount of oxygen from the maternal blood [78].

Diagnosis of delayed villous maturation becomes objective with the use of CD-15—*For the first time,* the current study has found that delayed villous maturation was present in 18.6 % (62/333) of spontaneous preterm labor cases. Although delayed villous maturation is also subjective and prone to much inter-observer variability [52, 72, 84, 86-88], the diagnosis of the lesion in the current study was based on CD-15 immunohistochemical staining. CD-15 is an immunohistochemical marker for villous immaturity and chronic placental dysfunction, thereby enhancing objective interpretation of this disorder of villous maturation [34, 35, 47, 72, 73, 85, 89-91]. Significant elevation in immature CD15+ endothelial cells in the macro- (chorionic plate and stem villous vessels) and micro-vasculature (terminal villous vessels), a type of endothelial immunophenotypic transformation, is deemed diagnostic for delayed villous maturation [34, 35, 72, 73, 85, 89, 90].

CD-31 staining in the current study was utilized as a quality–control technique [36]. CD-31 expression in the endothelial cells of the placenta is stable, consistently expressed by all the placental vessels throughout the pregnancy, and it is not affected by villous maturation abnormalities [34, 35, 37]. By contrast, CD-15 marks physiologically immature placentas of the first and second trimesters and pathologically immature term placentas [34, 35]. As compared to CD-31, a non-specific endothelial marker [36], CD-15 is a specific immunohistochemical marker for villous endothelial immaturity [34, 35] (Figure 4).

Clinical significance of disorders of villous maturation in spontaneous preterm labor—The placenta undergoes profound developmental changes during its lifespan, characterized by progressive accentuation in the number of villous capillaries [92] that culminates in the formation of vasculo-syncytial membranes [13-16]. These vasculo-syncytial membranes in the terminal villi shorten the diffusion distance between

the maternal and fetal circulation, thereby increasing oxygen diffusion capacity by 30-fold [93], and constitute the principal sites of gaseous exchange in the placenta [15, 17, 18]. Diminution in the number of vasculo-syncytial membranes suggests either villous immaturity or villous regressive changes [19]; furthermore, a reduced vasculo-syncytial membrane count has been implicated in a high incidence of hypoxic complications [19]. Defective maturation of the villous tree, therefore, can cause a paucity of vasculo-syncytial membranes resulting in placental dysfunction and fetal hypoxia [20-25, 73, 85]. Indeed, our group has recently demonstrated that placental delayed villous maturation is associated with evidence of chronic fetal hypoxia and with a higher concentration of amniotic fluid erythropoietin than in controls without delayed villous maturation [85]. This observation suggests that the structural abnormalities in the vasculo-syncytial membranes may lead to impaired exchange and clinical fetal hypoxemia in a fraction of the cases [85].

Acute placental inflammatory lesions—The frequency of maternal inflammatory response and fetal inflammatory response was significantly higher, and the frequencies of these lesions were inversely related to the gestational age at delivery (Table 4). Our findings are consistent with several other studies [94-98]. The frequency of acute placental inflammtion in extreme prematurity (<28 weeks of gestational age) was highest in this study (84.3% [54/64]) compared to the other categories of preterm birth, and this evidence also corroborates with previous literature detailing increased acute inflammation/ chorioamnionitis in earlier preterm births [62, 94, 99-101].

Chronic inflammatory placental lesions—The frequency of chronic placental lesions was the highest between 28 and 33 weeks of gestation and decreased gradually during early and late gestation, depicting an inverted U-shaped distribution (Figure 3). Chronic inflammatory placental lesions are relatively common at term with limited involvement and have been reported to be immunologic inflammatory lesions, similar to transplantation rejection and graft-versus-host disease [95, 102-106]. Our findings corroborate with prior reports that have identified a high frequency of chronic placental inflammation in preterm births [94, 101, 103, 107].

Lesions of maternal vascular malperfusion—The frequency of lesions of maternal vascular malperfusion was higher in cases than in controls and increased gradually as the period of gestational age at delivery decreased. Such lesions in spontaneous preterm labor have been reported previously [12, 95, 108-110]. Our findings of persistent muscularization of basal plate arteries (or failure of physiologic transformation of the spiral arteries) and mural hypertrophy of decidual arterioles in spontaneous preterm birth are congruent with earlier studies [12, 95, 108-115].

Placental histological patterns suggestive of fetal hypoxia—Histological patterns other than those described above included nucleated red blood cells [23, 116-118], hypercapillarized villi [23, 119-121], intravillous hemorrhage [122, 123], massive perivillous fibrinoid deposition [23, 49, 119, 124-128], and laminar necrosis of the decidua capsularis [129, 130]. Nucleated red blood cells, hypercapillarized villi, and intravillous

hemorrhage represent compensatory changes as a result of fetal hypoxia while massive perivillous fibrinoid deposition and laminar necrosis represent cellular hypoxia.

The presence of nucleated red blood cells in preterm labor has previously been reported by Roescher et al [131, 132]; however, the current study is the first to report nucleated red blood cells in spontaneous preterm labor. In the current study, nucleated red blood cells were observed in 1.1% (5/437) of spontaneous preterm labor cases but not in the control placentas with a normal pregnancy outcome [0% (0/942)]. The gestational age ranged from 21 weeks to 34.4 weeks.

Fetal hypoxemia-induced stimulation, production, and secretion of increased erythropoietin is understood to be the major underlying factor leading to elevated production and premature release of immature erythrocyte precursors [116-118, 133-137]. Elevated fetal plasma and amniotic fluid levels of erythropoietin are considered to represent true fetal hypoxemia given that erythropoietin does not cross the placenta and is not stored [118, 138]. A higher concentration of amniotic fluid erythropoietin has recently been demonstrated by our investigators to be associated with delayed villous maturation [85].

Hypercapillarization was observed in 15.6% (68/435) of placentas with spontaneous preterm labor versus 3.5% (33/938) of placentas with normal pregnancy outcome. Hypercapillarization has not been reported in previous spontaneous preterm labor studies.

Long-term exposure of the placenta to hypoxic conditions [139, 140] has been proposed to result in the hypercapillarization of villi and is generally assumed to result from impaired uteroplacental blood flow [23, 121]. This process is expressed as capillary profiles per villous profile [120]. It has been demonstrated that both mild and persistent hypoxia/ischemia enhance paracrine stimulation of endothelial cells by vascular endothelial growth factor via the kinase insert domain-containing receptor within the villous blood vessels, inducing angiogenesis and resulting in villous hypercapillarization [141].

Intravillous hemorrhage, chorionic villous hemorrhage, or villous stromal hemorrhage was observed in 20.1% (42/209) of the placentas with spontaneous preterm labor as compared to none in the normal outcome group [0% (0/944)].

Intravillous hemorrhage was first described in association with retroplacental hemorrhage [122], and it is considered to be a manifestation of an acute hypoxic event of the placenta [77], a consequence of hypoxic necrosis of the villous vessel with subsequent loss of vascular integrity and stromal hemorrhage [122]. Intravillous hemorrhage is more common and pronounced in early gestation, probably as a result of the relative fragility of the vessels and the loss of stroma [45]. The increased prevalence of intravillous hemorrhage in spontaneous preterm birth cases observed in the current study may be a result of the fragile and immature villous capillary vessels. Theoretically, it can also occur when the pressure in the villi becomes elevated above the threshold to cause villous capillary rupture [45]. This is the first study to report intravillous hemorrhage in placentas of spontaneous preterm labor. It is noteworthy that nucleated red blood cells, hypercapillarization, and intravillous hemorrhage are more frequent in fetal death than in spontaneous preterm labor [73].

Comprehending histopathologic findings in spontaneous preterm labor

Spontaneous preterm birth has survival value and is adaptive in nature—The placental histopathological lesions observed in the current study indicate that preterm labor has survival value as proposed by earlier studies [142-145]. This concept indicates that spontaneous preterm birth may be associated with lower rates of complications than induced preterm birth [142, 145]. In fact, it has been shown that the frequency of neonatal respiratory distress syndrome is greater following “indicated” preterm birth than spontaneous preterm birth [142]. Moreover, the "adaptive nature” of the clinical manifestation has been proposed in the context of preterm labor with intrauterine infection [145]. The onset of preterm labor may be considered a host-defense mechanism against intrauterine infection whereby the mother eliminates infected tissues (membranes, decidua, and/or fetus) to maintain reproductive fitness [145]. The survival value of premature labor may also be significant because it allows the fetus to escape a hostile intrauterine environment [145]. Indeed, fetuses who are born dead have a significantly higher frequency of disorders of villous maturation, placental histological patterns suggestive of hypoxia, than fetuses that are live-born in spontaneous preterm labor and delivery [73].

Preterm birth and long-term maternal cardiovascular risk—Several studies have reported that women with preterm birth experience higher blood pressure decades after delivery, including those with prior normotensive preterm birth compared to term birth [146]. It has been demonstrated that women who subsequently deliver preterm have higher atherogenic lipids and excessive inflammation during pregnancy compared to those who deliver at term [147-151]. Moreover, women who undergo early (<34 weeks of gestation) preterm birth show a more atherogenic lipid profile measured in the decade after delivery [152]. Preterm births with concurrence of malperfusion and infection/inflammation have been associated with smaller infant size, earlier delivery, and an excess of neonatal intraventricular hemorrhage [99]. Moreover, placentas with evidence of multiple pathological processes may also mark women as susceptible to a higher cardiometabolic burden detectable within the decade after pregnancy [153]. Patients with disorders of villous maturation, especially delayed villous maturation, along with hypoxic patterns of placental injury, have not been described earlier in spontaneous preterm labor and warrant follow-up studies.

Strengths and Limitations

The strengths of this study comprise 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; 5) the adaptation of definitions of disorders of villous maturation as discussed in German literature; and 6) the comparison of placental findings in spontaneous preterm labor with placentas from normal term controls.

The limitations of the study include the following: 1) participants were enrolled across different research protocols over an extended period of time and thus were not consecutively enrolled; 2) a lack of objective criteria in the diagnosis of accelerated villous maturation

does not allow for the comparison of current results to those of other researchers; 3) a lack of follow-up beyond the neonatal period; and 4) a lack of gestational age-matched controls.

Conclusion

Disorders of villous maturation are present in nearly one-third of the cases of spontaneous preterm labor. Spontaneous preterm labor is a syndrome that occurs by several pathogenic mechanisms involving abnormal placental villous development, hypoxic injury, placental vascular disease, acute placental inflammation, and maternal anti-fetal rejection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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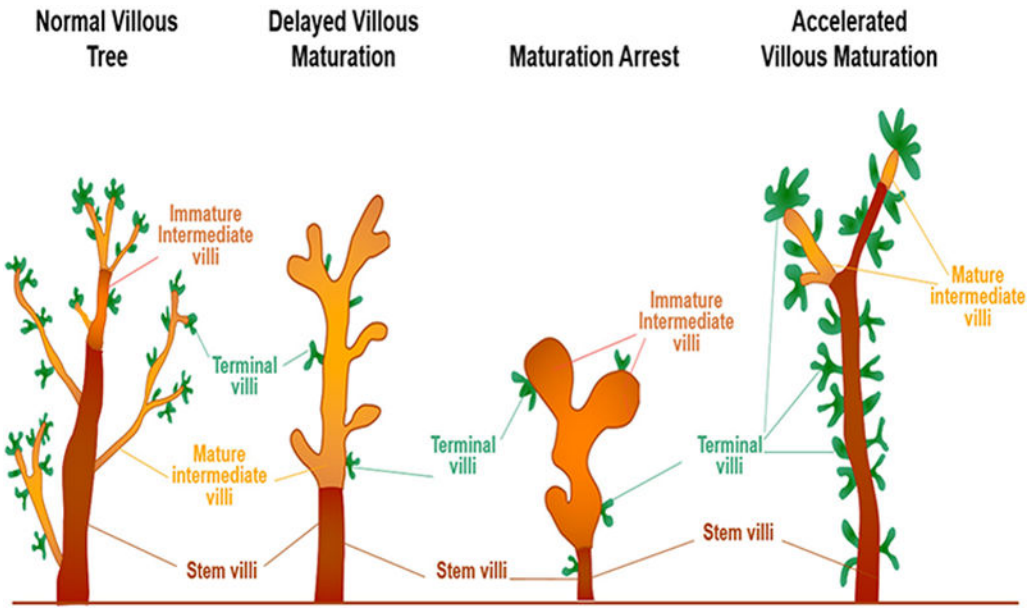

Figure 1:

Schematic representation of normal 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 (92) (0-5%; not depicted here). Delayed villous maturation is characterized by medium sized intermeidate 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 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, slender terminal villi resembling the histology of term villi with considerably increased intervillous space. (Reproduced with permission)

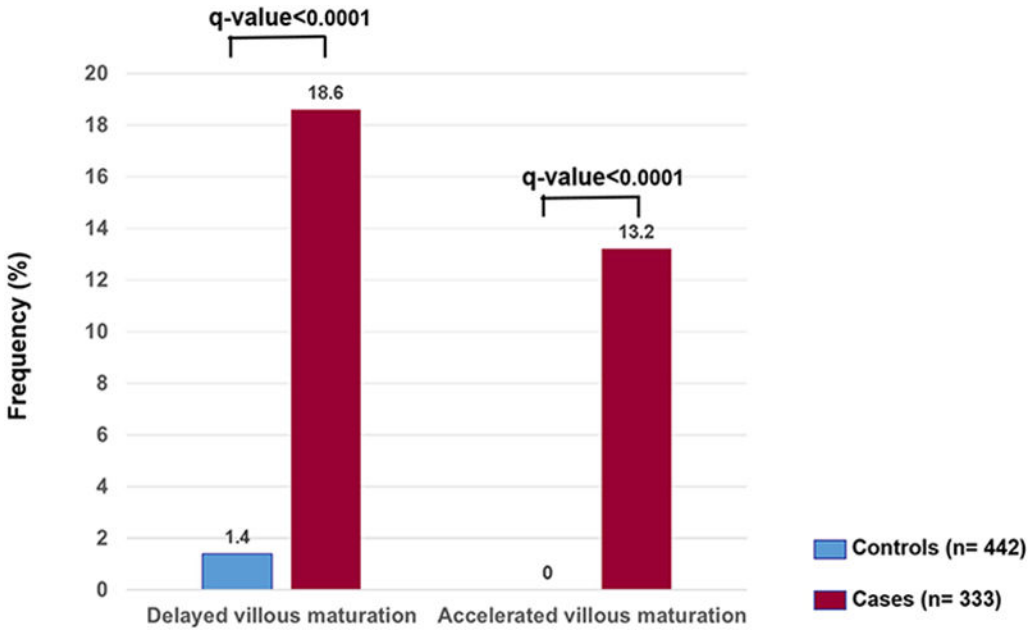

Figure 2:

Delayed villous maturation in cases 18.6% [62/333] vs. controls 1.4% [6/442], q <0.0001, prevalence ratio 13.7; and accelerated villous maturation in cases 13.2% [44/333] vs. controls 0% [0/442], q <0.001.

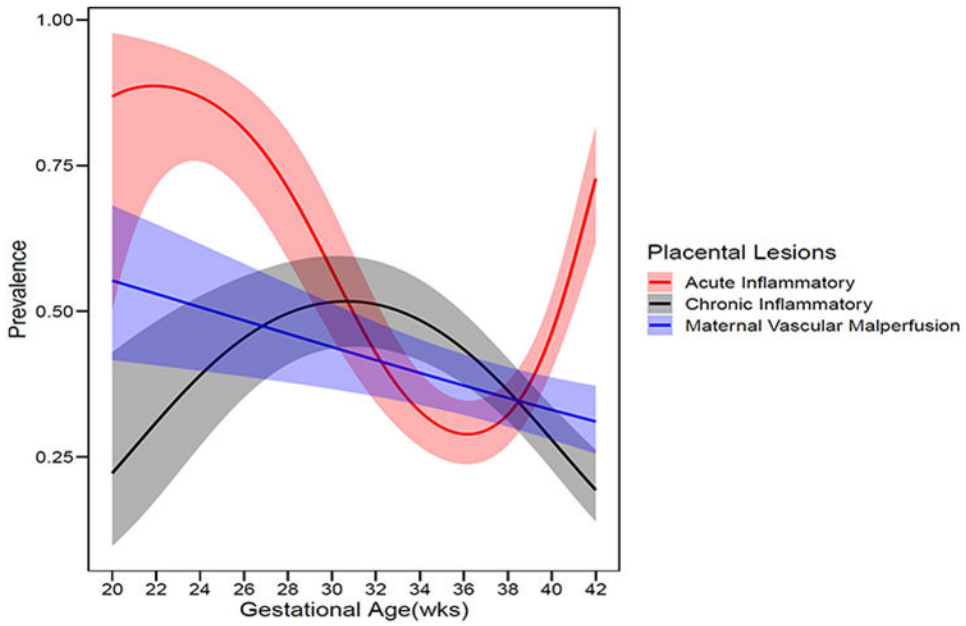

Figure 3:

The rate of acute placental lesions was inversely related to gestational age. Acute placental inflammatory lesions gradually increased as the period of gestational age at delivery decreased. On the other hand, the frequency of chronic placental lesions for a gestational age > 28 weeks was higher than those < 28 weeks. The frequency of chronic placental lesions was the highest between 28 and 33 weeks of gestation and decreased gradually during early and late gestation, depicting an inverted “U” shape distribution. The frequency of lesions of maternal vascular malperfusion was higher at a gestational age <28 weeks [53.1% (34/64)] than > 28 weeks [43% (161/374)].

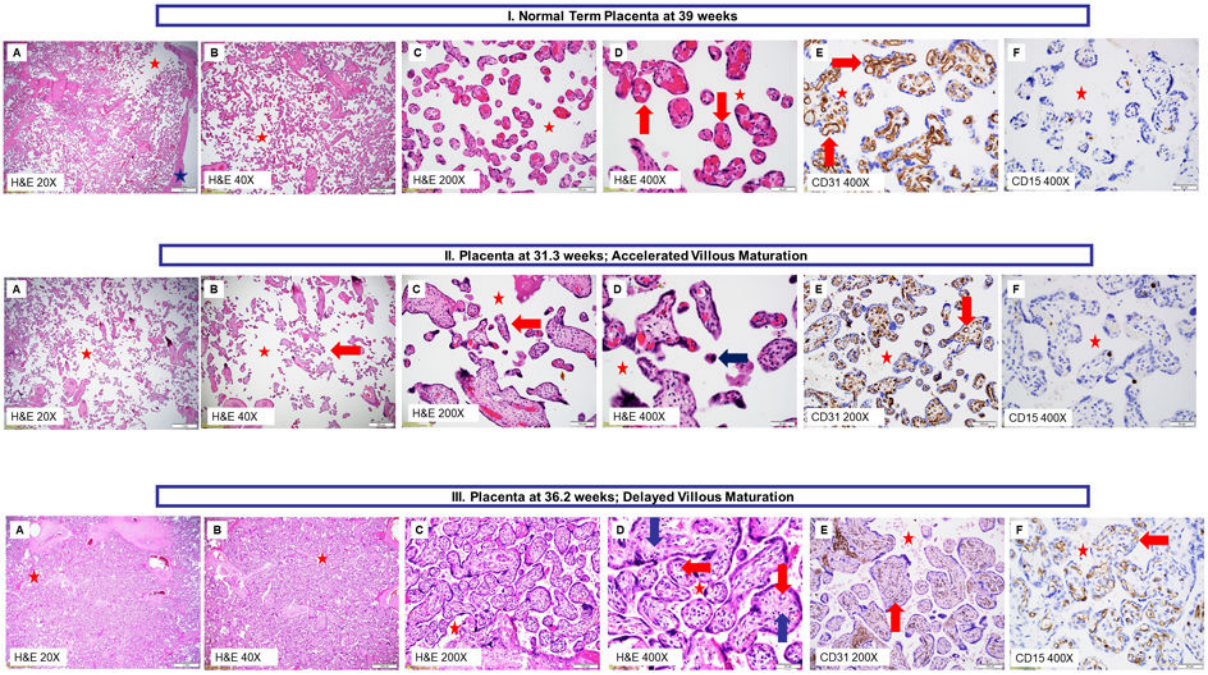

Figure 4:

Panel I: Normal term placenta (39 weeks). (A) Normal basal plate (blue star) with suprabasal chorionic villi that are mature and appropriate for gestational age; (A–D) normal mature well vascularized terminal villi; (D) Mature terminal villi with conspicuous capillaries, barely discernible stroma; arrows pointing towards vasculo-syncytial membranes formed by the apposition of trophoblasts with villous capillary endothelium (normal 3–5 per terminal villi); (E) CD-31 which stains all blood vessels, mature or immature, highlights villous capillaries and vasculo-syncytial membranes (brown stain; red arrows); (F) CD-15 which stains immature villous capillaries is negative in chorionic villous vessel endothelium (absence of brown staining). (A–D) H&E–20X, 40X, 200X; (E) CD-31-400X; (F) CD-15: 400X; red starintervillous space which is white. Panel II: Placenta at gestational age 31.3 weeks showing accelerated villous maturation. (A–D) 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; the terminal villi appear slender, hypermature, hypoplastic (red arrows). (D) Hypermature villi with villous syncytial knotting (blue arrow). (E) CD-31 highlighting villous capillaries which are predominantly central in location with relative paucity and inconspicuousness of vasculo-syncytial membranes (red arrow). (F) CD-15 negative chorionic villous capillary endothelium (absence of brown staining). (A–D) H&E–20X, 40X, 200X; (E) CD-31-200X; (F) CD-15: 400X. Panel III: Placenta at gestational age 36.2 weeks showing delayed villous maturation. (A–D) Chorionic villi appear crowded, immature, affecting >50% of placental villous population and resembling the histology of second trimester villi. Intervillous space is considerably diminished to virtually absent (red star). (D) The villi display more stroma (blue star), centralized vessels (red arrows) and paucity of vasculo-syncytial membranes as compared to normal pregnancy (refer to panel I). (E) CD-31 highlighting villous capillaries which are central in location with paucity of vasculo-syncytial membranes (red arrow). (F) CD15-positive

(brown staining) capillary endothelium in mature intermediate chorionic villi. (A–D) H&E–20X, 40X, 200X; (E) CD-31-200X; (F) CD-15: 400X.

Table 1.

Maternal characteristics and perinatal outcomes in normal pregnancies (controls) and pregnancies with preterm labor (cases)

	Controls (n=944)	Cases (n=438)	p-value
Maternal characteristics			
Maternal age (years)	24.8 (15-42)	24.3 (15-44)	0.09
Nulliparity	320 (33.9%)	117 (26.7%)	<0.01
Maternal height (cm)	163.1 (137.2-185.4)	162.1 (132.1-185.4)	<0.01
Pre-pregnancy BMI (kg/m^2)	27.2 (15.5-67.4)	26 (15.3-54.3)	<0.01
Underweight	30/886 (3.4%)	26/415 (6.3%)	0.02
Obese	257/886 (29%)	96/415 (23.1%)	0.02
Ethnicity			0.01
African American	764/943 (81%)	381 (87%)	
Caucasian	83/943 (8.8%)	32 (7.3%)	
Others	96/943 (10.2%)	25 (5.7%)	
Tobacco use during pregnancy	111/940 (11.8%)	96/437 (22%)	<0.0001
Alcohol use during pregnancy	7/939 (0.7%)	15/437 (3.4%)	<0.001
Drug use during pregnancy	118/942(12.5%)	97 (22.1%)	<0.0001
History of preterm delivery	68 (7.2%)	167/438 (38.1%)	<0.0001
Perinatal outcomes			
Gestational age at delivery (weeks)	39.6 (37-42)	33.2 (20-36.9)	<0.0001
Cesarean delivery	247 (26.2%)	80 (18.3%)	0.001
Induction of labor	137 (14.5%)	0 (0%)	<0.0001
Augmented labor	274 (29%)	81 (18.5%)	<0.0001
Spontaneous labor	408 (43.2%)	371 (84.7%)	<0.0001
Birth weight (g)	3345.7 (2560-4090)	2123.1 (247-4080)	<0.0001
Birth weight $<3^{rd}$ centile	0 (0%)	6 (1.4%)	<0.001
Ponderal index at birth (g/cm^3)	2.6 (1.5-11)	2.4 (1.2-8.7)	<0.0001
$<10^{th}$ percentile	122/864(14.1%)	58/407 (14.3%)	1
Male sex neonate’	486/940 (51.7%)	227/435 (52.2%)	0.9
Composite neonatal morbidity [a]	10 (1.1%)	184 (42%)	<0.0001
Early neonatal death	0/944 (0%)	32 (7.3%)	<0.0001

Data are presented as median (range) or n/N (%)

BMI, body mass index

p-value between normal pregnancy and pregnancy with preterm labor

[a]Composite neonatal morbidity includes 5-minute Apgar score<7, bronchopulmonary dysplasia, pulmonary hypoplasia, respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukomalacia, retinopathy of prematurity, neonatal sepsis, or NICU admission

Table 2.

Placentas on gross examination in normal pregnancies and pregnancies with preterm labor

Characteristics	Controls (n=944)	Cases (n=438)	p-value
Placental weight, untrimmed (g)	571.2(324-989)	445.4 (123-1211)	<0.0001
Placental weight < 10th percentile	224/930 (24.1%)	45/399(15.2%)	<0.0001
Fetoplacental weight ratio[a]	6.0 (3-10.2)	4.7 (1.5-8.5)	<0.0001
Placental disk dimensions			
Placental disc length (cm)	18.3 (12-32)	16.5 (10-25.5)	<0.0001
Placental disc width (cm)	15.7 (5.2-22)	14.4 (7-21)	<0.0001
Placental thickness (cm)	2.4 (0.5-4)	2.2 (0.6-4)	<0.0001
Placental volume (cm^3)	689.6 (150-1840)	542.4 (104-1449)	<0.0001
Umbilical cord			
Umbilical cord insertion			
Distance (cm)	5.5 (0.3-10)	4.7 (0-11)	<0.0001
Marginal insertion	11/938 (1.2%)	12/434 (2.8%)	0.04

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

[a]Value obtained dividing birthweight by placental weight. There were 943 placental weight in normal pregnancy and 438 palcental weight in pregnancy with preterm labor.

Table 3.

Frequency of placental histologic lesions in normal pregnancy and spontaneous preterm labor

Histologic lesions	Normal pregnancy (n=944)	Pregnancy with preterm labor (n=438)	q-value	Prevalence ratio
Acute inflammatory lesions	399 (42.3%)	202 (46.1%)	0.26	1.1
Maternal inflammatory response	341 (36.1%)	195 (44.5%)	< 0.005	1.2
Stage 1: early acute subchorionitis or chorionitis	220 (23.3%)	62 (14.2%)	<0.001	0.6
Stage 2: acute chorioamnionitis	114 (12.1%)	101 (23.1%)	<0.0001	1.9
Stage 3: necrotizing chorioamnionitis	7 (0.7%)	32 (7.3%)	<0.0001	9.9
Grade 2 (severe): Subchorionic microabscess	7 (0.7%)	34 (7.8%)	<0.0001	10.5
Fetal inflammatory response	219 (23.2%)	132 (30.1%)	<0.05	1.3
Stage 1: chorionic vasculitis or umbilical phlebitis	177 (18.8%)	56 (12.8%)	0.01	0.7
Stage 2: umbilical arteritis	41 (4.3%)	67 (15.3%)	<0.0001	3.5
Stage 3: necrotizing funisitis	1 (0.1%)	9 (2.1%)	<0.001	19.4
Grade 2 (severe): intense chorionic and/or umbilical vasculitis	2 (0.2%)	16 (3.7%)	<0.0001	17.2
Chronic inflammatory lesions	282 (29.9%)	210 (47.9)	<0.0001	1.6
Chronic deciduitis	182 (19.3%)	147 (33.6%)	<0.0001	1.7
Lymphocytic (without plasma cells)	97 (10.3%)	27 (6.2%)	0.03	0.6
Lymphoplasmacytic	85 (9%)	120 (27.4%)	<0.0001	3
Villitis of unknown etiology (VUE)	175 (18.5%)	54 (12.3%)	<0.01	0.7
Low-grade lesions	121/925 (13.1%)	26 (5.9%)	<0.001	0.5
High-grade lesions	11/925 (1.2%)	4 (0.9%)	0.90	0.8
Chronic chorioamnionitis	120 (12.7%)	128 (29.2%)	<0.0001	2.3
Grade 1/ stage 1	34/940 (3.6%)	66 (15.1%)	<0.0001	4.2
Grade 2/ stage 1	62/940 (6.6%)	35 (8%)	0.46	1.2
Grade 1/ stage 2	2/940 (0.2%)	6 (1.4%)	0.02	6.4
Grade 2/ stage 2	10/940 (1.1%)	16 (3.7%)	0.006	3.4
Chronic histiocytic intervillositis	4/942(0.4%)	0/436 (0%)	0.42	0
Villitis of infectious origin	0/942(0%)	0/436 (0%)	1	0
Eosinophilic T-cell vasculitis	4/942 (0.4%)	4/437 (0.9%)	0.38	2.2
Severe chronic inflammatory lesions	23/921(2.5%)	24/438 (5.5%)	0.01	2.2
Maternal vascular malperfusion	337 (35.7%)	195 (44.5%)	0.006	1.2
Villous Changes	176 (18.6%)	89 (20.3%)	0.57	1.1
Villous infarct(s)	53/943 (5.6%)	16 (3.7%)	0.22	0.6
Increased syncytial knots	80 (8.5%)	29 (6.6%)	0.38	0.8
Villous agglutination	19 (2%)	4 (0.9%)	0.26	0.5
Increased intervillous fibrin deposition	60 (6.4%)	54 (12.3%)	0.001	1.9
Distal villous hypoplasia	1 (0.1%)	6 (1.4%)	0.01	12.9
Vascular Lesions	194 (20.6%)	134 (30.6%)	<0.001	1.5
Persistent muscularization of basal plate arteries	154 (16.3%)	101 (23.1%)	<0.01	1.4
Mural hypertrophy of decidual arterioles	17 (1.8%)	20 (4.6%)	0.01	2.5

Histologic lesions	Normal pregnancy (n=944)	Pregnancy with preterm labor (n=438)	q-value	Prevalence ratio
Acute atherosis of basal plate arteries and/or decidual arterioles	8 (0.8%)	6 (1.4%)	0.49	1.6
Spiral artery fibrinoid necrosis	3/942 (0.3%)	6/436 (1.4%)	0.06	4.3
Decidual vascular thrombosis	16/942 (1.7%)	15/436 (3.4%)	0.09	2
Persistence of endovascular trophoblast	3/942 (0.3%)	5/436 (1.1%)	0.18	3.6
Retroplacental hemorrhage	8/942 (0.8%)	5/436 (1.1%)	0.67	1.4
≥ 2 lesions of maternal vascular malperfusion	70 (7.4%)	57 (13%)	<0.01	1.8
≥ 3 lesions of maternal vascular malperfusion	11 (1.2%)	11/438 (2.5%)	0.11	2.2
Fetal vascular malperfusion	186 (19.7%)	76 (17.4%)	0.44	0.9
Villous Changes	107 (11.3%)	38 (8.7%)	0.24	0.8
Villous stromal-vascular karyorrhexis	24 (2.5%)	6 (1.4%)	0.33	0.5
Hyalinized avascular villi, small foci	62 (6.6%)	16 (3.7%)	0.06	0.6
Hyalinized avascular villi, variable sized foci	19 (2%)	16 (3.7%)	0.16	1.8
Fetal thrombotic vasculopathy	2 (0.2%)	0 (0%)	1.0	
Vascular Lesions	85 (9%)	43 (9.8%)	0.73	1.1
Thrombi in large fetal vessels	25 (2.6%)	15 (3.4%)	0.59	1.3
Intimal fibrin deposition, large fetal vessels	61 (6.5%)	31 (7.1%)	0.84	1.1
≥ 2 lesions of fetal vascular malperfusion	7 (0.7%)	8 (1.8%)	0.15	2.5
Hypoxic histologic patterns of placental injury				
Nucleated red blood cells	0 (0%)	5/437 (1.1%)	<0.01	
Hypercapillarized villi	33/938 (3.5%)	68/435 (15.6%)	<0.0001	4.4
Massive perivillous fibrin deposition	2/942 (0.2%)	1/436 (0.2%)	1.00	1.1
Laminar necrosis of the decidua capsularis	52/942 (5.5%)	25/436 (5.7%)	1	1

Table 4.

Frequency of placental histologic lesions in normal pregnancy and spontaneous preterm labor at different gestational age periods

Histologic lesions	Normal pregnancy (n=944)	Preterm Labor <24 weeks (n=30)	Preterm Labor 24-27 weeks (n=34)	Preterm Labor 28-32 weeks (n=53)	Preterm Labor 32-37 weeks (n=321)	q-value
Acute inflammatory lesions	399 (42.3%)	28 (93.3%)[a]	26 (76.5%)[b]	33 (62.3%)[b]	115 (35.8%)[c]	<0.0001
Maternal inflammatory response	341 (36.1%)	28 (93.3%)[a]	26 (76.5%)[a]	31 (58.5%)[a]	110 (34.3%)	<0.0001
Stage 1: early acute subchorionitis or chorionitis	220 (23.3%)	7 (23.3%)	8 (23.5%)	4 (7.5%)[a]	43 (13.4%)[c]	<0.001
Stage 2: acute chorioamnionitis	114 (12.1%)	11 (36.7%)[b]	7 (20.6%)	22 (41.5%)[a]	61 (19%)	<0.0001
Stage 3: necrotizing chorioamnionitis	7 (0.7%)	10 (33.3%)[a]	11 (32.4%)[a]	5 (9.4%)[a]	6 (1.9%)	<0.0001
Grade 2 (severe): Subchorionic microabscess	7 (0.7%)	12 (40%)[a]	11 (32.4%)[a]	5 (9.4%)[a]	6 (1.9%)	<0.0001
Fetal inflammatory response	219 (23.2%)	16 (53.3%)[b]	14 (41.2%)	29 (54.7%)[a]	73 (22.7%)	<0.0001
Stage 1: chorionic vasculitis or umbilical phlebitis	177 (18.8%)	9 (30%)	5 (14.7%)	8 (15.1%)	34 (10.6%)[b]	<0.01
Stage 2: umbilical arteritis	41 (4.3%)	5 (16.7%)[c]	6 (17.6%)[c]	19 (35.8%)	37 (11.5%)	<0.0001
Stage 3: necrotizing funisitis	1 (0.1%)	2 (6.7%)[c]	3 (8.8%)[b]	2 (3.8%)[b]	2 (0.6%)	<0.0001
Grade 2 (severe): intense chorionic and/or umbilical vasculitis	2 (0.2%)	3 (10%)[b]	4 (11.8%)[a]	4 (7.5%)[a]	5 (1.6%)	<0.0001
Chronic inflammatory lesions	282 (29.9%)	9 (30%)	17 (50%)	28 (52.8%)[a]	156 (48.6%)[a]	<0.0001
Chronic deciduitis	182 (19.3%)	9 (30%)	15 (44.1%)[b]	20 (37.7%)[b]	103 (32.1%)[a]	<0.0001
Lymphocytic (without plasma cells)	97 (10.3%)	3 (10%)	4 (11.8%)	4 (7.5%)[c]	16 (5%)	0.07
Lymphoplasmacytic	85 (9%)	6 (20%)	11 (32.4%)[b]	16 (30.2)[a]	87 (27.1%)[a]	<0.0001
Villitis of unknown etiology (VUE)	175 (18.5%)	0 (0%)	0 (0%)	3 (5.7%)[b]	51 (15.9%)[b]	<0.0001
Low-grade lesions	121 (13.1%)	0 (0%)	0 (0%)	2 (3.8%)[b]	24 (7.5%)	<0.001
High-grade lesions	11 (1.2%)	0 (0%)	0 (0%)	0 (0%)	4 (1.2%)	1.00
Chronic chorioamnionitis	120 (12.7%)	2 (6.7%)	14 (41.2%)[a]	17 (32.1%)[a]	95 (29.6%)[a]	<0.0001
Stage 1/Grade 1	34 (3.6%)	1 (3.3%)	3 (8.8%)	10 (18.9%)[a]	52 (16.2%)[a]	<0.0001
Stage 2/ Grade 1	62 (6.6%)	1 (3.3%)	5 (14.7%)	3 (5.7%)	26 (8.1%)	0.46

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Histologic lesions	Normal pregnancy (n=944)	Preterm Labor <24 weeks (n=30)	Preterm Labor 24-27 weeks (n=34)	Preterm Labor 28-32 weeks (n=53)	Preterm Labor 32-37 weeks (n=321)	q-value
Stage 1/ Grade 2	2 (0.2%)	0 (0%)	2 (5.9%)	0 (0%)	4 (1.2%)	0.01
Stage 2/ Grade 2	10 (1.1%)	0 (0%)	4 (11.8%)	3 (5.7%)[b]	9 (2.8%)	0.001
Chronic histiocytic intervillositis	4/942(0.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.83
Villitis of infectious origin	0/942(0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.00
Eosinophilic T-cell vasculitis	4/942 (0.4%)	0 (0%)	0 (0%)	2 (3.8%)[c]	2/320 (0.6%)	0.18
Severe chronic inflammatory lesions	23/921(2.5%)	0 (0%)[b]	6 (17.6%)	3 (5.7%)	15 (4.7%)	0.002
Maternal vascular malperfusion	337 (35.7%)	15 (50%)	19 (55.9%)	23 (43.4%)	138 (43%)	0.02
Villous Changes	176 (18.6%)	5 (16.7%)	9 (26.5%)	11 (20.8%)	64 (19.9%)	0.88
Villous infarct(s)	53/943(5.6%)	0 (0%)	0 (0%)	0 (0%)	16 (5%)	0.27
Increased syncytial knots	80 (8.5%)	1 (3.3%)	1 (2.9%)	3 (5.7%)	24 (7.5%)	0.87
Villous agglutination	19 (2%)	0 (0%)	0 (0%)	1 (1.9%)	3 (0.9%)	0.83
Increased intervillous fibrin deposition	60 (6.4%)	4 (13.3%)	8 (23.5%)[b]	8 (15.1%)[c]	34 (10.6%)	<0.001
Distal villous hypoplasia	1 (0.1%)	0 (0%)	0 (0%)	2 (3.8%)[c]	4 (1.2%)	0.01
Vascular Lesions	194 (20.6%)	13 (43.3%)[c]	14 (41.2%)[c]	17 (32.1%)[c]	90 (28%)	<0.001
Persistent muscularization of basal plate arteries	154 (16.3%)	10 (33.3%)	9 (26.5%)	14 (26.4%)	68 (21.2%)	0.02
Mural hypertrophy of decidual arterioles	17 (1.8%)	3 (10%)	2 (5.9%)	3 (5.7%)	12 (3.7%)	0.01
Acute atherosis of basal plate arteries and/or decidual arterioles	8 (0.8%)	1 (3.3%)	1 (2.9%)	0 (0%)	4 (1.2%)	0.34
Spiral artery fibrinoid necrosis	3/942(0.3%)	0 (0%)	0 (0%)	1 (1.9%)	5 (1.6%)	0.15
Decidual vascular thrombosis	16/942(1.7%)	2 (6.7%)	1 (2.9%)	2 (3.8%)	10 (3.1%)	0.15
Persistence of endovascular trophoblast	3/942(0.3%)	2 (6.7%)	1 (2.9%)	0 (0%)	2 (0.6%)	0.01
Retroplacental hemorrhage	8/942(0.8%)	2 (6.7%)	1 (2.9%)	0(0%)	2 (0.6%)	0.08
Massive perivillous fibrin deposition	2/942(0.2%)	0 (0%)	0 (0%)	0 (0%)	1 (0.3%)	1.00
≥ 2 lesions of maternal vascular malperfusion	70 (7.4%)	8 (26.7%)	5 (14.7%)	8 (15.1%)	36 (11.2%)	<0.01
≥ 3 lesions of maternal vascular malperfusion	11 (1.2%)	1 (3.3%)	0 (0%)	2 (3.8%)	8 (2.5%)	0.2
Fetal vascular malperfusion	186 (19.7%)	7 (23.3%)	4 (11.8%)	4 (7.5%)	61 (19%)	0.23
Villous Changes	107 (11.3%)	4 (13.3%)	1 (2.9%)	2 (3.8%)	31 (9.7%)	0.31
Villous stromal-vascular karyorrhexis	24 (2.5%)	1 (3.3%)	0 (0%)	0 (0%)	5 (1.6%)	0.72

Histologic lesions	Normal pregnancy (n=944)	Preterm Labor <24 weeks (n=30)	Preterm Labor 24-27 weeks (n=34)	Preterm Labor 28-32 weeks (n=53)	Preterm Labor 32-37 weeks (n=321)	q-value
Hyalinized avascular villi, small foci	62 (6.6%)	1 (3.3%)	0 (0%)	2 (3.8%)	13 (4%)	0.40
Hyalinized avascular villi, variable sized foci	19 (2%)	2 (6.7%)	1 (2.9%)	0 (0%)	13 (4%)	0.14
Fetal thrombotic vasculopathy	2 (0.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.00
Vascular Lesions	85 (9%)	3 (10%)	3 (8.8%)	2 (3.8%)	35 (10.9%)	0.70
Thrombi in large fetal vessels	25 (2.6%)	1 (3.3%)	1 (2.9%)	0 (0%)	13 (4%)	0.55
Intimal fibrin deposition, large fetal vessels	61 (6.5%)	2 (6.7%)	3 (8.8%)	2 (3.8%)	24 (7.5%)	0.91
≥ 2 lesions of fetal vascular malperfusion	7 (0.7%)	0 (0%)	1 (2.9%)	0 (0%)	7 (2.2%)	0.21
Hypoxic histologic patterns of placental injury						
Nucleated red blood cells	0 (0%)	3 (10%)	0 (0%)	1 (1.9%)	1 (0.3%)	<0.01
Hypercapillarized villi	33 (3.5%)	5 (16.7%)	6 (17.6%)	14 (26.4%)	43/318 (13.5%)	<0.0001
Massive Perivillous Fibrinoid Deposition	2 (0.2%)	0 (0%)	0 (0%)	0 (0%)	1 (0.3%)	1.0
Laminar necrosis of the decidua capsularis	52 (5.5%)	0 (0%)	2 (5.9%)	4 (7.5%)	19 (6%)	1.0

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

BMI, body mass index; NICU, neonatal intensive care unit.

[a] p <0.001 as compared to normal pregnancy

[b] p <0.01 as compared to normal pregnancy

[c] p value <0.05 as compared to normal pregnancy