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The role of the placenta in spontaneous preterm labor and delivery with intact membranes

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Abstract

Objectives: To determine whether placental vascular pathology and impaired placental exchange due to maturational defects are involved in the etiology of spontaneous

preterm labor and delivery in cases without histologic acute chorioamnionitis.

Methods: This was a retrospective, observational study. Cases included pregnancies that resulted in spontaneous preterm labor and delivery (<37 weeks), whereas uncomplicated pregnancies that delivered fetuses at term (≥ 37 –42 weeks of gestation) were selected as controls. Placental histological diagnoses were classified into three groups: lesions of maternal vascular malperfusion, lesions of fetal vascular malperfusion, and placental microvasculopathy, and the frequency of each type of lesion in cases and controls was compared. Moreover, we specifically searched for villous maturational abnormalities in cases and controls. Doppler velocimetry of the umbilical and uterine arteries were performed in a subset of patients.

Results: There were 184 cases and 2471 controls, of which 95 and 1178 had Doppler studies, respectively. The frequency of lesions of maternal vascular malperfusion was greater in the placentas of patients with preterm labor than in the control group [14.1% (26/184) vs. 8.8% (217/2471) ($p=0.023$)]. Disorders of villous maturation were more frequent in the group with preterm labor than in the control group: 41.1% (39/95) [delayed villous maturation in 31.6% (30/95) vs. 2.5% (13/519) in controls and accelerated villous maturation in 9.5% (9/95) vs. none in controls].

Conclusions: Maturational defects of placental villi were associated with approximately 41% of cases of unexplained spontaneous preterm labor and delivery without acute inflammatory lesions of the placenta and with delivery of appropriate-for-gestational-age fetuses.

Keywords: accelerated villous maturation; delayed villous maturation; Doppler study; fetoplacental weight ratio; maternal vascular malperfusion; placental microvasculopathy; pregnancy preterm birth; pulsatility index; umbilical artery; uterine artery.

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Introduction

Preterm birth is the leading cause of death in children younger than 5 years of age, accounting for 35% of deaths in neonates and affecting 10.6% of livebirths [1, 2].

Furthermore, those infants who survive have a higher rate of long-term morbidity, including neurologic and developmental disabilities [3] and a shorter life expectancy compared to infants born full term [4]. To develop effective preventive measures to reduce the incidence of preterm birth, there is a need to understand the causes of spontaneous preterm birth [2, 5].

There has been burgeoning interest to understand the mechanisms by which the placenta might cause pregnancy complications, such as fetal growth restriction [6–16], preeclampsia [7–9, 11, 12, 17–25], fetal death [10–12, 16, 26–29], and spontaneous preterm birth [8, 10–12, 30–33]. The main functions of the placenta are to provide nutrition for fetal growth and to serve as “the lung” of the fetus. These exchange functions depend on low impedance and high flow circulations on the maternal and fetal sides of the placenta. Therefore, an important pathway whereby the placenta causes non-infection/inflammation-related complications of pregnancy must involve abnormalities of the fetoplacental circulation that impair the exchange function of the placenta and, likely, increase placental vascular resistance, whatever the initiating cause(s) of these changes [34]. This is the case in fetal growth restriction, preeclampsia, and fetal death [35–41].

In this study, we examined the hypothesis that placental causes of spontaneous preterm labor and delivery interfere with the exchange function of the placenta by increasing placental vascular impedance and by impairing the placenta’s ability to meet the fetal demands for nutrients. We compared how placental vascular resistance, as reflected in the Doppler velocity waveform of the umbilical artery, changes with gestational age in normal term pregnancies and in otherwise normal pregnancies that delivered preterm. In addition, we examined the relationship between fetal and placental growth prior to delivery and placental vascular development and pathology. Given that abnormal development or maturation of the placental villous tree and its vasculature can affect placental exchange by decreasing the mass of terminal villi available for exchange [6, 42–44], we also examined the maturation of the placental villi in cases of preterm delivery for which Doppler studies were done during pregnancy.

Materials and methods

Study design

This was a retrospective observational study of structurally normal singleton pregnancies in women recruited onto cohort and cross-

sectional studies conducted at Hutzel Women’s Hospital, Detroit, Michigan, USA, between January 2008 and January 2016. From this cohort, we selected as cases pregnancies without other medical or obstetrical complications that resulted in spontaneous preterm labor and delivery. Uncomplicated pregnancies that delivered fetuses at term (≥ 37 –42 weeks of gestation) were selected as controls [45]. Exclusion criteria included maternal chronic conditions; preterm prelabor rupture of the membranes; acute inflammatory lesions of the placenta (histologic acute chorioamnionitis, funisitis, and acute chorionic vasculitis); multiple gestation; congenital anomalies; preeclampsia; gestational hypertension; HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome [33]; small for gestational age; large for gestational age; fetal death; sonographic short cervix; placenta previa; and placenta accreta.

Clinical information, results of Doppler studies, and histopathological findings were retrieved retrospectively from a database. All study participants provided written informed consent prior to sample collection, and the use of clinical data and biological specimens obtained from these women for research purposes was approved by the Institutional Review Boards of Wayne State University and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (NICHD/NIH/DHHS).

Clinical definitions

Gestational age was determined by the last menstrual period and confirmed by ultrasound examination, or by the crown-rump length measurement in the first trimester of pregnancy by ultrasound examination alone if the sonographic determination of gestational age was not consistent with menstrual dating [46].

Estimated fetal weight was calculated by using the Hadlock formula [47] based on measurements of fetal biparietal diameter, head circumference, abdominal circumference, and femur length.

Small-for-gestational-age neonate was defined as a neonate with a birth weight below the 10th percentile for gestational age, using U.S. customized birth weight standards [48].

Appropriate-for-gestational-age (AGA) neonate was defined as a neonate with a birth weight between the 10th and 90th percentiles, using U.S. customized birth weight standards [48].

Spontaneous preterm labor was diagnosed by the presence of the following criteria: (1) regular uterine contractions occurring at a frequency of 4 in 20 min for a minimum of 1 h before 37 completed weeks of gestation; (2) cervical dilatation of ≥ 2 cm and effacement of $\geq 80\%$; or (3) cervical change [49, 50].

Preterm delivery was defined as a birth occurring ≤ 37 weeks gestational age.

Doppler studies

Ultrasound and Doppler studies were performed by using the General Electric Voluson Expert and Voluson E8 (GE Healthcare, Milwaukee, WI, USA) ultrasound systems and 2–5 MHz probes. Doppler evaluation of the uterine arteries was performed in a parasagittal view of the uterus 1 cm above the virtual crossing with the iliac artery [51]. The mean uterine artery pulsatility index was estimated as the average of the left and right uterine arteries.

Doppler evaluation of the umbilical artery was performed in a free loop of the umbilical cord and a high-pass wall filter of 60 MHz. The size of the Doppler sample gate was adjusted to cover the entire

interrogated vessel, and Doppler recordings were obtained in the absence of maternal or fetal movements with an angle of insonation as close as possible to 0°.

Placental pathology examination

The processing of placentas and the preparation of blocks and histological slides have been previously described [45]. A minimum of six random samples was taken from the central portion of each placenta, following the protocol established by the Perinatology Research Branch [45]: three samples were embedded in paraffin and stained with hematoxylin and eosin (H&E), while the remaining three samples were stored for further research. Further targeted sampling with preparation of blocks and slides was performed according to the Wayne State University Department of Pathology protocol [45]. Slides were examined by six trained pediatric and placental pathologists. Placental lesions were classified into three categories: (1) lesions of maternal vascular malperfusion, (2) lesions of fetal vascular malperfusion, and (3) placental microvasculopathy, based on the involvement of placental, maternal, and fetal villous tissues. Details of the lesions are as follows:

- (1) **Maternal vascular malperfusion** included the lesions attributed to deficient spiral artery transformation: (i) persistent muscularization of the basal plate arteries; (ii) acute atherosclerosis of the basal plate arteries; (iii) mural hypertrophy of the decidual arteries; (iv) fibrinoid necrosis of the spiral arteries; (v) persistence of endovascular trophoblast; and (vi) spiral artery thrombosis [52–57].
- (2) **Fetal vascular malperfusion** included lesions attributed to an obstruction in the large fetal vessels (chorionic plate and stem villous vessels): (i) thrombi in large fetal vessels; (ii) intimal fibrin deposition in large fetal vessels; and (iii) fibromuscular sclerosis in intermediate-size vessels [52–59].
- (3) **Placental microvasculopathy** included lesions of maternal villi: (i) distal villous hypoplasia; (ii) villous infarcts; (iii) villous stromal-vascular karyorrhexis; (iv) hyalinized avascular villi; or (v) fetal thrombotic vasculopathy (average of 15 avascular villi per slide).

Assessment of villous maturation

Maturation disorders of the placenta are disorders of the villous tree whereby the maturation is not in accordance with the gestational age [33, 53, 60–74]. A key characteristic of maturational disorders is a diminution in the number of vasculo-syncytial membranes, the main mechanism by which the placenta meets growing fetal demand [75, 76], which has been implicated in a high incidence of hypoxic complications [53, 60, 66, 74–79].

Structural disturbances of villous maturation generally described include: (1) accelerated for gestational age or accelerated villous maturation [33, 53, 57, 61, 67, 80] and (2) immature for gestational age or delayed villous maturation [33, 52, 60, 62, 64, 66, 80, 81].

Accelerated villous maturation, also termed “villous hypermaturity”, is defined as premature maturation of terminal chorionic villi [53, 63, 71]. Key histologic features include increased syncytial knots, distal villous hypoplasia, and the presence of small for-gestational-age villi [53]. Based on the current accepted criteria, the diagnosis of accelerated villous maturation does not apply to term pregnancies [52, 53, 61, 82].

Delayed villous maturation is defined by the presence of monotonous immature villi in at least one-third of a slide. Hallmarks of immature villi include (1) the presence of centrally placed capillaries, (2) a relatively large amount of stroma, and (3) fewer and less well-formed vasculosyncytial membranes [53, 62, 64, 73, 80, 81].

To objectively diagnose delayed villous maturation, apart from routine H&E evaluation, we performed CD15 immunohistochemistry. Endothelium positivity by CD15 stain is diagnostic of villous immaturity [81, 86–89]. Previous studies proposed that both macrovasculature (chorionic plate and stem vessels) and microvasculature (terminal villi) expressing ≥50% positive CD15 stain are indicative of villous immaturity [33, 81, 86–90]. In our study, CD15 stain was used in all preterm cases (n=184) and in a subset of controls (n=519; out of total term controls n=2471).

Statistical analysis

Continuous variables were compared between groups by using the Student's t-Test, and categorical factors were compared by using the Fisher's exact test. Least squares regression was used to regress variables, such as the birth weight, placental weight, and fetoplacental weight ratio on gestational age, and the effect of covariates (maternal age, nulliparity, and villous maturational findings) on each regression was determined. Linear mixed effects models were used to do the same for longitudinal variables such as the estimated fetal weight, umbilical artery pulsatility index, and uterine artery pulsatility index. All data analyses were performed in R [91]. A p-value of less than 0.05 was considered statistically significant.

Results

Demographic characteristics

There were 184 cases of spontaneous preterm labor and delivery and 2471 controls. Doppler studies were available for 51.6% (95/184) and 47.7% (1178/2471) respectively. Demographic data for term and preterm deliveries with or without Doppler studies were compared to assess any selection biases that resulted by including only cases that had Doppler studies performed during pregnancy for the evaluation of placental maturational defects. A significantly higher proportion of controls with Doppler studies were nulliparous and had placental lesions of maternal vascular malperfusion, placental lesions of fetal vascular malperfusion, and placenta microvasculopathy compared to controls without Doppler studies; the controls with Doppler studies were also younger in age and had a higher maternal body mass index (Table 1).

Cases and controls also differed significantly on a number of factors. In addition to the expected differences in the mean age at delivery and in the gestational age-related differences in the mean birth weight, placental weight, and

fetoplacental weight ratio, significantly fewer cases than controls were nulliparous, and a significantly higher proportion were smokers and delivered male fetuses (Table 1).

Relationship between the pulsatility indices of the umbilical and uterine arteries and gestational age

The umbilical artery pulsatility index decreased throughout pregnancy in a nonlinear manner in cases and controls. The umbilical artery pulsatility index was consistently lower in male than in female fetuses ($p < 0.001$; Figure 1A), but there was no significant difference in the umbilical artery pulsatility index between cases and controls after adjusting for fetal gender and parity ($p = 0.98$; Figure 1B). In the cases, the umbilical artery pulsatility index of placentas with accelerated villous maturation was lower than that of placentas with delayed villous maturation and those with no maturation defects, but the difference did not reach statistical significance ($p = 0.22$; Figure 1C).

The uterine artery pulsatility index also decreased with gestational age in a nonlinear manner in cases and controls, although to a much lesser extent, there was no difference in the uterine artery pulsatility index and gestational-age relationship between cases and controls ($p = 0.58$; Figure 1D). In cases, the uterine artery pulsatility index of placentas with delayed villous maturation was lower than that of placentas with no maturation defects, but the difference did not reach statistical significance ($p = 0.10$; Figure 1E). Likewise, the uterine artery pulsatility index of placentas with accelerated villous maturation was not significantly different from that of placentas with no maturation defects ($p = 0.87$; Figure 1E).

Placental pathologic findings

The frequency of lesions of maternal vascular malperfusion was significantly higher in placentas of patients with preterm labor than in controls [14.1% (26/184) vs. 8.8% (217/2471); ($p = 0.02$)] (Table 1). Maturation defects were present in the placentas of 41.1% (39/95) of patients with premature labor who had Doppler studies performed, whereas in only 2.5% (13/519) of controls. The most common maturational defect in patients with preterm labor was delayed villous maturation [31.6% (30/95)] assessed by CD15 staining, followed by accelerated villous maturation (9.5% (9/95)] (Figure 2).

Birth weight to placental weight ratio and fetoplacental weight ratio

In cases, the birth weight, placental weight and fetoplacental weight ratio increased progressively throughout the third trimester of pregnancy. After adjusting for gestational age, nulliparity, and fetal sex, there was no difference in expected birth weight in the preterm groups according to villous maturation (delayed villous maturation vs. normal maturation $p = 0.32$; accelerated villous maturation vs. normal maturation $p = 0.35$; Figure 3A). Cases with accelerated villous maturation had significantly lower mean placental weight than cases with normal maturation ($p = 0.004$; Figure 3B). The difference between the mean placental weight of cases with delayed villous maturation and those with normal maturation was statistically insignificant ($p = 0.89$; Figure 3B).

The mean birth weight to placental weight ratio was significantly higher in patients with preterm labor and histological evidence of accelerated villous maturation than in those with preterm labor/delivery and normal maturation ($p = 0.003$; Figure 3C). There was no difference in the mean birth weight to placental weight ratio in patients with preterm labor and histological evidence of delayed villous maturation compared to those with normal maturation ($p = 0.91$; Figure 3C). At an earlier gestational age (< 30 weeks), the estimated fetal weight of controls was significantly higher than that of gestational age-matched birth weight in spontaneous preterm labor and delivery (Figure 3D).

Discussion

Principal findings of the study

(1) The frequency of lesions of maternal vascular malperfusion was greater in the placentas of patients with preterm labor than in the control group [14.1% (26/184) vs. 8.8% (217/2471), $p = 0.02$]; (2) disorders of villous maturation were more frequent in the group with preterm labor than in the term control group [total 41.1% (39/95); delayed villous maturation 31.6% (30/95) vs. 2.5% (13/519) in controls; and accelerated villous maturation (9.5% (9/95)]; and (3) there were no differences in the umbilical artery and uterine artery pulsatility indices between cases and controls after adjusting for gestational age, fetal gender, and parity.

Table 1: Demographic data of the placental pathologic findings for term and preterm deliveries with or without Doppler studies.

	Term delivery			Preterm delivery			Term delivery vs. preterm delivery		
	Doppler (n=1,178)	No Doppler (n=1,293)	p-Value	Doppler (n=95)	No Doppler (n=89)	p-Value	Term delivery (n=2,471)	Preterm delivery (n=184)	p-Value
Age	23.53 (15–41)	24.29 (15–44)	0.0003	24.52 (16–41)	23.54 (15–38)	0.2105	23.93 (15–44)	24.04 (15–41)	0.7812
Body mass index	26.85 (14.4–61.3)	26.02 (15.5–64.1)	0.0026	25.9 (17–54.3)	24.93 (15.3–51)	0.3401	26.43 (14.4–64.1)	25.43 (15.3–54.3)	0.0589
Smoking status	180/1,172 (15.4%)	194/1,291 (15%)	0.8223	20/95 (21.1%)	20/89 (22.5%)	0.8593	374/2,463 (15.2%)	40/184 (21.7%)	0.0265
Nulliparity	390/1,178 (33.1%)	500/1,293 (38.7%)	0.0043	20/95 (21.1%)	27/89 (30.3%)	0.1770	890/2,471 (36%)	47/184 (25.5%)	0.0039
Birth weight	3,283.3 (2270–4,445)	3,291.8 (2390–4,335)	0.5367	2,572.82 (881–3,315)	2,441.25 (725–3,170)	0.0512	3,287.75 (2270–4,445)	2509.18 (725–3,315)	0.0000
Placental weight	561.17 (289–1,020)	565.02 (218–1,027)	0.3841	503.54 (268–840)	480.44 (197–790)	0.1398	563.18 (218–1,027)	492.36 (197–840)	0.0000
Birth weight to placental weight ratio	6 (3.77–12.36)	5.98 (2.97–13.35)	0.5937	5.23 (2.31–9.28)	5.16 (3.09–7.24)	0.6310	5.99 (2.97–13.35)	5.2 (2.31–9.28)	0.0000
Male neonates	595/1,178 (50.5%)	654/1,289 (50.7%)	0.9357	57/95 (60%)	51/88 (58%)	0.8805	1,249/2,467 (50.6%)	108/183 (59%)	0.0317
Gestational age at delivery (calculated)	39.48 (37–42.1)	39.51 (37–42.6)	0.4517	35.37 (26.9–36.9)	35.06 (25.1–36.9)	0.3191	39.5 (37–42.6)	35.22 (25.1–36.9)	0.0000
Maternal vascular malperfusion	141/1,178 (12%)	76/1,293 (5.9%)	0.0000	12/95 (12.6%)	14/89 (15.7%)	0.6727	217/2,471 (8.8%)	26/184 (14.1%)	0.0232
Fetal vascular malperfusion	48/1,178 (4.1%)	28/1,293 (2.2%)	0.0070	1/95 (1.1%)	0/89 (0%)	1.0000	76/2,471 (3.1%)	1/184 (0.5%)	0.0407
Placental microvasculopathy	122/1,178 (10.4%)	99/1,293 (7.7%)	0.0199	8/95 (8.4%)	5/89 (5.6%)	0.5696	221/2,471 (8.9%)	13/184 (7.1%)	0.4993

n, total number.

Figure 1: Gestational age-dependent differences in the mean umbilical artery pulsatility index and mean uterine artery pulsatility index between different study groups.

(A) Gestational age-dependent differences in the mean umbilical artery pulsatility index between multiparous females who delivered a male versus a female neonate at term. The umbilical artery pulsatility index was consistently lower in male than in female fetuses ($p < 0.001$). (B) Gestational age-dependent differences in the mean umbilical artery pulsatility index between multiparous women carrying a female fetus who delivered at term (controls) versus those who delivered preterm (cases). After adjusting for fetal gender and parity, there was no significant difference in umbilical artery pulsatility index between preterm cases and term controls ($p = 0.98$). (C) Gestational age-dependent differences in the mean umbilical artery pulsatility index in disorders of villous maturation in multiparous women carrying a female fetus who delivered preterm. The umbilical artery pulsatility index of placentas with accelerated villous maturation was lower than that of placentas with delayed villous maturation and those with no maturation defects, but the difference did not reach statistical significance ($p = 0.22$). (D) Gestational age-dependent differences in the mean uterine artery pulsatility index between multiparous women carrying a female fetus who delivered at term (controls) versus those who delivered preterm (cases). There was no difference in the uterine artery pulsatility index and gestational-age relationship between preterm cases and term controls ($p = 0.58$). (E) Gestational age-dependent differences in the mean uterine artery pulsatility index in disorders of villous maturation in multiparous women carrying a female fetus who delivered preterm. The uterine artery pulsatility index of placentas with delayed villous maturation was lower than that of placentas with no maturation defects, but the difference did not reach statistical significance ($p = 0.10$).

The development of the villous tree and placental angiogenesis

Blood flow in the umbilical arteries normally increases steadily until term as impedance in the fetoplacental circulation continually decreases [6, 42–44]. This decrease in impedance depends on carefully timed development of the placental villous tree, which grows and develops until term and provides a structural framework within which fetal

vessels can develop [34, 92, 93]. Vascularization begins with the *de novo* formation of blood vessels from mesenchymal precursors (vasculogenesis) in the first trimester. During the second and third trimesters of pregnancy, new blood vessels are formed from the existing ones (angiogenesis). Angiogenesis occurs in two ways, referred to as branching or non-branching [34, 92–95]. In branching angiogenesis, new vessels sprout from existing ones, whereas in non-branching angiogenesis, capillary loops form by elongation

Figure 2: Placental histopathology of normal term placentas as compared to placentas showing disorders of villous maturation — accelerated and delayed. Panel I: Normal term placenta (39 weeks). (A–C) Normal chorionic villi that are mature and appropriate for gestational age with normal intervillous space (white space; red star); (C) Mature terminal villi with arrows pointing towards vasculo-syncytial membranes formed by the apposition of syncytiotrophoblasts with villous capillary endothelium (normal 3–5 per terminal villi); terminal villi with conspicuous capillaries and barely discernible stroma; (D) chorionic villous capillary endothelium negative for CD-15 staining (absence of brown staining). (A–C) H&E-100X, 200X and 400X; (D) CD-15-100X. Panel II: Placenta at gestational age 32.2 weeks showing accelerated villous maturation. (A–C) 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, and hypoplastic (red arrows). (C) Hypermature villi with villous syncytial knotting (blue arrow). (D) CD-15 negative chorionic villous capillary endothelium (absence of brown staining). (A–C) H&E-100X, 200X and 400X; (D) CD-15-100X. Panel III: Placenta at gestational age 36.4 weeks showing delayed villous maturation. (A–C) Chorionic villi appear crowded and 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). 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). (D) CD15-positive (brown staining) capillary endothelium in mature intermediate chorionic villi and stem villi. (A–C) H&E-100X, 200X and 400X; (D) CD-15-100X.

of existing capillaries [34, 92, 93, 95–100]. Angiogenic factors are implicated in the regulation and type of angiogenesis. These growth factors include 1) vascular endothelial growth factor (VEGF), with its two receptor-type tyrosine kinases, Flt-1 (VEGF receptor-1) and KDR/Flk-1 (VEGF receptor-2), and 2) placental growth factor (PlGF) and its receptor Flt-1 (VEGFR-1) [93, 101, 102]. Of note, the expression of angiogenic factors is modulated by the partial pressure of oxygen (PO_2) in the placenta [102].

Mesenchymal villi, the progenitors of each type of chorionic villi, continue to form throughout pregnancy and start to vascularize between 5 and 6 weeks of

gestation [6, 34, 92]. During the first and second trimesters of pregnancy, they progressively differentiate into immature intermediate villi [92–94] that, in turn, differentiate into stem villi [6, 34, 92, 93]. The process of mesenchymal villi differentiation into immature intermediate villi changes at about 23–24 weeks of gestation when mesenchymal villi transform into mature intermediate villi [34]. From then on, placental vascular development continues by non-branching rather than branching angiogenesis [6, 34, 92]. Mature intermediate villi do not differentiate into stem villi or branch-like immature intermediate villi; instead, terminal villi are

Figure 3: The mean birth weight (A), the mean placental weight (B), and the mean birth weight to placental weight ratio (C) by gestational age-stratified according to the disorders of villous maturation in multiparous women who delivered preterm.

(A) There was no difference in expected birth weight in the preterm groups in relation to villous maturation (delayed villous maturation vs. normal maturation $p=0.32$; and accelerated villous maturation vs. normal maturation $p=0.35$); (B) the mean placental weight for cases with accelerated villous maturation was significantly lower than for cases with normal maturation ($p=0.004$); however, the mean placental weight for delayed villous maturation cases and normal maturation did not show any difference ($p=0.89$); (C) the mean fetoplacental weight ratio was significantly higher in patients with preterm labor and histological evidence of accelerated villous maturation than in those with normal maturation ($p=0.003$). However, there was no difference in the mean fetoplacental weight ratio in patients with preterm labor and histological evidence of delayed villous maturation than in those with normal maturation ($p=0.91$). (D) Gestational age-dependent differences between the mean estimated fetal weight of term female neonates and the mean birth weights of preterm female neonates stratified according to the disorders of villous maturation in multiparous women. At an earlier gestation (<30 weeks), the estimated fetal weight for controls was significantly higher than that of gestational age-matched birthweight in spontaneous preterm labor and deliveries. The significance of differences in mean birth weights of preterm neonates and the gestational age-matched mean estimated fetal weights of term fetuses was judged by examining the overlaps between the confidence intervals.

formed passively on their surfaces by the differential growth rate of the mature intermediate villi capillaries and the trophoblast that covers them [6, 34, 92, 93]. Capillaries in mature intermediate villi grow longitudinally and more rapidly than the overlying trophoblast, become coiled as a result, and thin out and push the trophoblast into the intervillous space as protuberances that are the terminal villi, the placenta's most efficient gas exchange units [101, 102]. Placental growth then slows, but the placenta continues to develop by producing a large number of terminal villi [93–95].

This pattern of vascular development has recently been challenged by the findings derived from three-

dimensional reconstructions of terminal villi in term placentas [103]. Jirkovska et al. [103] observed three categories of terminal villi: those that protrude from existing terminal villi, those that protrude from mature intermediate villi, and those that are completely separated from mature intermediate villi but connected by capillary beds to the vessels in the mature intermediate villi. The investigators also observed two patterns of capillary development: by linear growth and elongation of capillaries and by sprouting from existing capillaries [103]. These findings indicate that branching angiogenesis is not confined to immature intermediate villi and that it also takes place in mature intermediate villi.

It follows from Poiseuille's law that the impedance to flow of vessels produced by branching angiogenesis will be lower than the impedance of vessels produced by linear growth (non-branching angiogenesis), as impedance is directly proportional to the length of the vessel, and branching angiogenesis reduces the overall length of individual vessels spanning a given distance between two points [104, 105]. Therefore, the impedance of the fetoplacental circulation should vary with the proportion of vessels in the placental vascular tree produced by branching and non-branching angiogenesis in the second and third trimesters of pregnancy. Since the umbilical artery pulsatility index reflects impedance of the fetoplacental circulation, the relationship between the umbilical artery pulsatility index and gestational age potentially provides a surrogate measure of how the placental vasculature is developing and whether it is developing differently in pregnancies that deliver at term or preterm.

Disorders of placental villous maturation in spontaneous preterm labor: evidence suggesting an impaired placental exchange with normal vascular impedance

An overarching hypothesis in obstetrics is that placental causes of pregnancy complications (fetal growth restriction, preeclampsia, and fetal death) are mediated by abnormalities of the placental vasculature that impair the exchange function of the placenta. The findings reported herein indicated that, in spontaneous preterm labor, placental vascular resistance, as reflected in the umbilical artery pulsatility index, does not increase, and the placental lesions observed consist largely of a maturational defect of the villous tree that may impair the exchange function of the placenta.

Given that the relationship between the umbilical artery pulsatility index and gestational age for cases and controls was superimposable and that the estimated fetal weight for controls was also almost identical to gestational age-matched birth weight among cases, impaired placental function would seem at first blush not to be implicated in preterm labor. However, impaired placental exchange was implicated in 41.1% (39/95) of cases by virtue of the presence of delayed villous maturation or accelerated villous maturation in the placenta. Maturation defects can be expected to impair placental exchange by reducing the mass of terminal villi available for exchange, without increasing placental impedance, because branching angiogenesis is normal in these cases but for the fact that it continues beyond (in delayed villous maturation) or terminates before (in accelerated villous maturation) the point in gestation

when transition to non-branching angiogenesis is believed to occur normally [6, 93, 95, 96, 99, 106–108]. In addition, delayed villous maturation has been associated with stillbirths [28, 29, 60, 68, 80, 109, 110] and accelerated villous maturation with placental lesions of maternal vascular malperfusion [63, 111]. Accelerated villous maturation cannot be detected in term controls by the accepted current definition [52, 53, 74] and delayed villous maturation could be detected only in a small minority of controls (2.5%; 13/519), so maturational defects appear to sufficiently contribute to uncomplicated preterm labor and delivery in 41% (39/95) of cases.

The frequency of disorders of villous maturation in this study is consistent with an earlier report that has shown disorders of villous maturation are present in nearly one-third of the cases of spontaneous preterm birth (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], $q < 0.001$) [33].

Our findings were consistent with those of Morgan et al. [61] who reported a higher frequency of accelerated villous maturation in cases of “idiopathic” spontaneous preterm birth than in those associated with acute histological chorioamnionitis [84% (26/31) vs. 30% (10/33) $p = < 0.001$]. The frequency of maturational disorders is similar to that observed in patients with preeclampsia and fetal growth restriction. Collectively, the available data suggest that disorders of villous maturation, implicated in the placental exchange function, are associated with preterm deliveries. We previously reviewed the evidence that vascular disorders are implicated in the etiology of preterm labor [112].

Mechanisms whereby disorders of villous maturation impair the exchange function of the placenta

Dilated capillaries are thought to occupy more than 35% of the total villous volume at term [105] and, therefore, could provide a significant enhancement to fetal oxygen uptake. In an image-based study of blood flow and oxygen transfer in placental capillaries [113], it has been shown that a localized dilation of optimal shape of the villous capillaries was found to increase oxygen transfer by up to 15%. The model supports the hypothesis that localized villous dilations develop toward term to enhance oxygen transfer in the placenta without radical placental growth or remodeling. In the second half of gestation, placental growth increases at a slower rate than fetal growth [114], while the

mean trophoblast thickness decreases until term [115] to improve oxygen exchange.

The results of this modelling study also provide an explanation for the hypoxia-related outcomes of delayed villous maturation [62]. The lack of vasculosyncytial membranes observed in cases of delayed villous maturation could represent a lack of localized fetal dilations and, therefore, lead to the inability of the fetus to extract an appropriate amount of oxygen from the maternal blood [113].

In summary, inadequate oxygen or nutrient transfer in the placenta can be secondary to primary lesions of the villi, which decrease the diffusion capacity of the vasculosyncytial membranes, or to impaired maternal blood flow [114, 117]. In our study, we could not find evidence of impaired impedance to flow in the umbilical artery and the abnormal findings were largely due to disorders of villous maturation.

Clinical implications

If an impaired exchange function of the placenta is a cause of preterm labor, and if birth weight and placental weight can be taken as reasonable surrogate variables for fetal demand and placental supply, then the trigger for preterm delivery does not appear to be a current mismatch between fetal demand and placental supply, i.e., mismatch at the time of delivery. The reasons are two-fold: (1) the birth weight of fetuses delivered preterm was not significantly different from gestational age-matched estimated fetal weight for AGA neonates born at term; and (2) the mean birth weight to placental weight ratio or the fetoplacental weight ratio was also not significantly different between the different subgroups of spontaneous preterm labor and delivery, notwithstanding that gestational age-adjusted placental weight was lower and that the gestational age-adjusted birth weight to placental weight ratio or the fetoplacental weight ratio was higher for cases of accelerated villous maturation than for the other two preterm delivery subgroups. These findings suggest that placental supply was within adequate limits for fetal demand at the time of delivery in these cases, whether or not maturational defects were present.

Therefore, if a mechanism exists to trigger preterm labor that is linked to the exchange efficiency of the placenta, the critical signal is likely to be a mismatch between prospective fetal demand relative to prospective placental supply. This, in turn, implies that the critical signal is the relative rates at which birth weight and placental weight are increasing with gestational age (“growth rate ratio”), which could signal

that future fetal demand will outstrip placental supply and trigger preterm labor. However, since the fetal growth rate among preterm and term deliveries was similar, this also implies that the factor triggering preterm delivery is a decline in the rate of placental growth. Unfortunately, we were unable to test this hypothesis in the current study as the fetoplacental ratio varies with gestational age, and placental weight cannot be estimated *in utero* at the present time. Nonetheless, from an evolutionary standpoint, such a mechanism would maximize the mother’s ability to pass on her genes by maximizing her chances for delivering a viable fetus.

In the human evolutionary past, when average life expectancy was considerably shorter than the time span of fertility, females had a limited number of opportunities to produce a live offspring and would derive a competitive advantage in two ways from having a mechanism that detected impaired placental development. During the first half of pregnancy, detection of impaired placental growth would allow the mother to terminate a pregnancy that was unlikely to yield a viable offspring and to start afresh, instead of investing limited fertility time in a pregnancy that was not going to pass on her genes. At the other end of pregnancy, once the fetus has reached a stage at which it had a good chance of survival in the outside world, the same monitoring system, with its gain reset to a higher level, would allow the mother to deliver prior to term if placental growth fell below a critical level such that future fetal demands were unlikely to be met. This would protect the fetus from the potentially life-threatening situation that could develop if the mother went into labor with a fetus whose demands, especially for oxygen, were barely being met, and that could not easily withstand the acute interruptions of placental supply that occur during labor. Indeed, the fact that preterm labor is 50–100 times more common than stillbirth could be explained on the basis of such a protective mechanism.

Nevertheless, our hypothesis needs to be validated, and this will require the comparison of fetal growth and placental growth *in utero* prior to birth. However, if confirmed, the proposed signal would explain the many diverse situations in which preterm delivery occurs, especially in the absence of pathological or functional (i.e., fetal growth restriction) indices of placental insufficiency; it is entirely possible for a situation to arise in which fetal growth and placental growth are both within normal limits, yet the rate at which the fetus is growing outstrips the rate of placental growth such that fetal needs will not be met by the placenta prior to term.

Conclusions

Maturation defects of placental villi can contribute to unexplained spontaneous preterm labor and delivery of AGA fetuses in cases that do not present with acute inflammatory lesions of the placenta.

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References

1. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Global Health* 2019;7:e37–46.
2. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science* 2014;345:760–5.
3. Marlow N, Wolke D, Bracewell MA, Samara M. Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med* 2005;352:9–19.
4. Crump C. An overview of adult health outcomes after preterm birth. *Early Hum Dev* 2020;150:105187.
5. Romero R, Kusanovic JP, Chaiworapongsa T, Hassan SS. Placental bed disorders in preterm labor, preterm PROM, spontaneous abortion and abruptio placentae. *Best Pract Res Clin Obstet Gynaecol* 2011;25:313–27.
6. Kingdom J, Huppertz B, Seaward G, Kaufmann P. Development of the placental villous tree and its consequences for fetal growth. *Eur J Obstet Gynecol Reprod Biol* 2000;92:35–43.
7. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol Reprod* 2003;69:1–7.
8. Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. *Placenta* 2009;30: S43–8.
9. Kim JY, Kim YM. Acute atherosclerosis of the uterine spiral arteries: clinicopathologic implications. *J Pathol Trans Med* 2015;49: 462–71.
10. Kim YM, Chaemsathong P, Romero R, Shaman M, Kim CJ, Kim JS, et al. The frequency of acute atherosclerosis in normal pregnancy and preterm labor, preeclampsia, small-for-gestational age, fetal death and midtrimester spontaneous abortion. *J Matern Fetal Neonatal Med* 2015;28:1–9.
11. Kim YM, Chaemsathong 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.
12. Korzeniewski SJ, Romero R, Chaiworapongsa T, Chaemsathong P, Kim CJ, Kim YM, et al. Maternal plasma angiogenic index-1 (placental growth factor/soluble vascular endothelial growth factor receptor-1) is a biomarker for the burden of placental lesions

- consistent with uteroplacental underperfusion: a longitudinal case-cohort study. *Am J Obstet Gynecol* 2016;214:629.
13. Kingdom JC, Audette MC, Hobson SR, Windrim RC, Morgen E. A placenta clinic approach to the diagnosis and management of fetal growth restriction. *Am J Obstet Gynecol* 2018;218: S803–17.
 14. Gibbs I, Leavey K, Benton SJ, Gynspan D, Bainbridge SA, Cox BJ. Placental transcriptional and histological subtypes of normotensive fetal growth restriction are comparable to preeclampsia. *Am J Obstet Gynecol* 2019;220:110.e1–e21.
 15. Kovo M, Schreiber L, Ben-Haroush A, Wand S, Golan A, Bar J. Placental vascular lesion differences in pregnancy-induced hypertension and normotensive fetal growth restriction. *Am J Obstet Gynecol* 2010;202:561.
 16. Pacora P, Romero R, Jung E, Gudicha DW, Hernandez-Andrade E, Musilova I, et al. Reduced fetal growth velocity precedes antepartum fetal death. *Ultrasound Obstet Gynecol* 2021;57: 942–52.
 17. Espinoza J, Romero R, Mee Kim Y, Kusanovic JP, Hassan S, Erez O, et al. Normal and abnormal transformation of the spiral arteries during pregnancy. *J Perinat Med* 2006;34:447–58.
 18. Ogge G, Chaiworapongsa T, Romero R, Hussein Y, Kusanovic JP, Yeo L, et al. Placental lesions associated with maternal underperfusion are more frequent in early-onset than in late-onset preeclampsia. *J Perinat Med* 2011;39:641–52.
 19. Redman CW, Sargent IL, Staff AC. IFPA Senior Award Lecture: making sense of pre-eclampsia - two placental causes of preeclampsia? *Placenta* 2014;35:S20–5.
 20. Brosens I, Brosens JJ, Muter J, Puttemans P, Benagiano G. Preeclampsia: the role of persistent endothelial cells in uteroplacental arteries. *Am J Obstet Gynecol* 2019, <https://doi.org/10.1016/j.ajog.2019.01.239>.
 21. Benton SJ, Leavey K, Gynspan D, Cox BJ, Bainbridge SA. The clinical heterogeneity of preeclampsia is related to both placental gene expression and placental histopathology. *Am J Obstet Gynecol* 2018, <https://doi.org/10.1016/j.ajog.2018.09.036>.
 22. Redman CW, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. *Am J Obstet Gynecol* 2015;213(4 Suppl):S9–11.
 23. Fisher SJ. Why is placental abnormal in preeclampsia? *Am J Obstet Gynecol* 2015;213(4 Suppl):S115–22.
 24. Quinn MJ. Preeclampsia: 2 placental phenotypes, 1 etiology? *Am J Obstet Gynecol* 2014;211:313–4.
 25. Nelson DB, Ziadie MS, McIntire DD, Rogers BB, Leveno KJ. Placental pathology suggesting that preeclampsia is more than one disease. *Am J Obstet Gynecol* 2014;210:66.
 26. Maiti K, Sultana Z, Aitken RJ, Morris J, Park F, Andrew B, et al. Evidence that fetal death is associated with placental aging. *Am J Obstet Gynecol* 2017;217:441.
 27. Chaiworapongsa T, Romero R, Erez O, Tarca AL, Conde-Agudelo A, Chaemsaitong P, et al. The prediction of fetal death with a simple maternal blood test at 24–28 weeks: a role for angiogenic index-1 (PIGF/sVEGFR-1 ratio). *Am J Obstet Gynecol* 2017;217:682.
 28. 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.
 29. Jaiman S, Romero R, Pacora P, Jung E, Bhatti G, Yeo L, et al. Disorders of placental villous maturation in fetal death. *J Perinat Med* 2020;48:345–68.
 30. Kim YM, Chaiworapongsa T, Gomez R, Bujold E, Yoon BH, Rotmensch S, et al. Failure of physiologic transformation of the spiral arteries in the placental bed in preterm premature rupture of membranes. *Am J Obstet Gynecol* 2002;187:1137–42.
 31. Kim YM, Bujold E, Chaiworapongsa T, Gomez R, Yoon BH, Thaler HT, et al. Failure of physiologic transformation of the spiral arteries in patients with preterm labor and intact membranes. *Am J Obstet Gynecol* 2003;189:1063–9.
 32. Kim CJ, Romero R, Kusanovic JP, Yoo W, Dong Z, Topping V, et al. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. *Mod Pathol* 2010;23:1000–11.
 33. Jaiman S, Romero R, Pacora P, Erez O, Jung E, Tarca AL, et al. Disorders of placental villous maturation are present in one-third of cases with spontaneous preterm labor. *J Perinat Med* 2021;49:412–30.
 34. Castellucci M, Kosanke G, Verdenelli F, Huppertz B, Kaufmann P. Villous sprouting: fundamental mechanisms of human placental development. *Hum Reprod Update* 2000;6:485–94.
 35. Aurioles-Garibay A, Hernandez-Andrade E, Romero R, Qureshi F, Ahn H, Jacques SM, et al. Prenatal diagnosis of a placental infarction hematoma associated with fetal growth restriction, preeclampsia and fetal death: clinicopathological correlation. *Fetal Diagn Ther* 2014;36:154–61.
 36. Chaiworapongsa T, Chaemsaitong P, Yeo L, Romero R. Preeclampsia part 1: current understanding of its pathophysiology. *Nat Rev Nephrol* 2014;10:466–80.
 37. Chaiworapongsa T, Romero R, Kusanovic JP, Savasan ZA, Kim SK, Mazaki-Tovi S, et al. Unexplained fetal death is associated with increased concentrations of anti-angiogenic factors in amniotic fluid. *J Matern Fetal Neonatal Med* 2010;23:794–805.
 38. Docheva N, Romero R, Chaemsaitong P, Tarca AL, Bhatti G, Pacora P, et al. The profiles of soluble adhesion molecules in the “great obstetrical syndromes”. *J Matern Fetal Neonatal Med* 2019;32:2113–36.
 39. Labarrere CA, DiCarlo HL, Bammerlin E, Hardin JW, Kim YM, Chaemsaitong P, et al. Failure of physiologic transformation of spiral arteries, endothelial and trophoblast cell activation, and acute atherosclerosis in the basal plate of the placenta. *Am J Obstet Gynecol* 2017;216:287.
 40. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, Divakar H, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: a pragmatic guide for first-trimester screening and prevention. *Int J Gynaecol Obstet* 2019; 145:1–33.
 41. Chaiworapongsa T, Kusanovic JP, Savasan ZA, Mazaki-Tovi S, Kim SK, Vaisbuch E, et al. Fetal death: a condition with a dissociation in the concentrations of soluble vascular endothelial growth factor receptor-2 between the maternal and fetal compartments. *J Matern Fetal Neonatal Med* 2010;23: 960–72.
 42. Adamson SL. Arterial pressure, vascular input impedance, and resistance as determinants of pulsatile blood flow in the umbilical artery. *Eur J Obstet Gynecol Reprod Biol* 1999;84: 119–25.

43. Fisk NM, MacLachlan N, Ellis C, Tannirandorn Y, Tonge HM, Rodeck CH. Absent end-diastolic flow in first trimester umbilical artery. *Lancet* (London, England) 1988;2:1256–7.
44. Hendricks SK, Sorensen TK, Wang KY, Bushnell JM, Seguin EM, Zingheim RW. Doppler umbilical artery waveform indices—normal values from fourteen to forty-two weeks. *Am J Obstet Gynecol* 1989;161:761–5.
45. 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.
46. Committee opinion No 700: methods for estimating the due date. *Obstet Gynecol.* 2017;129:e150–4.
47. Hadlock FP, Deter RL, Harrist RB, Park SK. Estimating fetal age: computer-assisted analysis of multiple fetal growth parameters. *Radiology* 1984;152:497–501.
48. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. *Obstet Gynecol* 1996; 87:163–8.
49. Guinn DA, Goldenberg RL, Hauth JC, Andrews WW, Thom E, Romero R. Risk factors for the development of preterm premature rupture of the membranes after arrest of preterm labor. *Am J Obstet Gynecol* 1995;173:1310–5.
50. Hassan S, Romero R, Hendler I, Gomez R, Khalek N, Espinoza J, et al. A sonographic short cervix as the only clinical manifestation of intra-amniotic infection. *J Perinat Med* 2006; 34:13–9.
51. Bhide A, Acharya G, Bilardo CM, Brezinka C, Cafici D, Hernandez-Andrade E, et al. ISUOG practice guidelines: use of Doppler ultrasonography in obstetrics. *Ultrasound Obstet Gynecol* 2013; 41:233–9.
52. 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.
53. Khong TY, Mooney EE, Nikkels PGJ, Morgan TK, Gordijn SJ. Pathology of the placenta: a practical guide. Switzerland AG: Springer Nature; 2019.
54. Redline RW. Classification of placental lesions. *Am J Obstet Gynecol* 2015;213(4 Suppl):S21–8.
55. Redline RW. The clinical implications of placental diagnoses. *Semin Perinatol* 2015;39:2–8.
56. Redline RW, Boyd TK, Roberts DJ. Placental and gestational pathology, Cohen MC, Scheimberg I, editors. Cambridge: Cambridge University Press; 2018.
57. Redline RW. Maternal vascular malperfusion. In: Redline RW, Boyd TK, Roberts DJ, editors. Placental and gestational pathology, 1st ed. United Kingdom: Cambridge University Press; 2018:62–9 pp.
58. Demir R, Kosanke G, Kohnen G, Kertschanska S, Kaufmann P. Classification of human placental stem villi: review of structural and functional aspects. *Microsc Res Tech* 1997;38:29–41.
59. Sati L, Demir AY, Sarikcioglu L, Demir R. Arrangement of collagen fibers in human placental stem villi. *Acta Histochem* 2008;110: 371–9.
60. 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.
61. 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.
62. 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.
63. Christians JKGD. Placental villous hypermaturation is associated with improved neonatal outcomes. *Placenta* 2019;76:1–5.
64. 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.
65. Kaufmann P, Luckhardt M, Schweikhart G, Cantle SJ. Cross-sectional features and three-dimensional structure of human placental villi. *Placenta* 1987;8:235–47.
66. Vogel M. Atlas der morphologischen plazentadiagnostik, 2nd ed. Berlin: Springer; 1996.
67. Fitzgerald BKJ, Keating S. Distal villous hypoplasia. *Diagn Histopathol* 2012;18:195–200.
68. 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.
69. Huynh J, Dawson D, Roberts D, Bentley-Lewis R. A systematic review of placental pathology in maternal diabetes mellitus. *Placenta* 2015;36:101–14.
70. 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.
71. Redline R. Maternal vascular malperfusion. In: Redline RW, Boyd TK, Roberts DJ, editors. Placental and gestational pathology. Diagnostic pediatric pathology. Cambridge: Cambridge University Press; 2018.
72. Schweikhart G, Kaufmann P, Beck T. Morphology of placental villi after premature delivery and its clinical relevance. *Arch Gynecol* 1986;239:101–14.
73. Treacy A, Higgins M, Kearney JM, McAuliffe F, Mooney EE. Delayed villous maturation of the placenta: quantitative assessment in different cohorts. *Pediatr Dev Pathol* 2013;16: 63–6.
74. Vogel M, Turowski G. Clinical pathology of the placenta. Berlin, Boston: Walter de Gruyter GmbH; 2019.
75. Benirschke K, Kaufmann P, Baergen RN. Pathology of the human placenta, 5th ed. New York: Springer-Verlag; 2006:1050 p.
76. Dimitrovska N. [Characteristics of the vasculosyncytial membranes in human chorioplacental villi]. *Jugosl Ginekolo Opstet* 1978;18:279–84.
77. Gordijn SJ, Korteweg FJ, Erwich JJ, Holm JP, Ravisé JM, Nikkels PG, Veeger NJ, Khong YT, Timmer A. Placental villus immaturity as an important cause of term fetal death. In: Fetal death: classification and diagnostic work-up. University of Groningen, Netherlands. 2010;99–110.
78. 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.
79. Jaiman S, Romero R, Pacora P, Jung EJ, Kacerovsky M, Bhatti G, et al. Placental delayed villous maturation is associated with evidence of chronic fetal hypoxia. *J Perinat Med* 2020;48: 516–8.

80. Redline RW. Distal villous immaturity. *Diagn Histopathol* 2012; 18:189–94.
81. 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.
82. Morgan TK. Role of the placenta in preterm birth: a review. *Am J Perinatol* 2016;33:258–66.
83. 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.
84. 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; 1990:81–113 pp.
85. Turowski G, Vogel M. Re-view and view on maturation disorders in the placenta. *APMIS (Acta Pathol Microbiol Immunol Scand)* 2018;126:602–12.
86. Seidmann L, Suhan T, Kamyschanskiy Y, Nevmerzhitskaya A, Gerein V, Kirkpatrick CJ. CD15 – a new marker of pathological villous immaturity of the term placenta. *Placenta* 2014;35:925–31.
87. 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.
88. 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.
89. Seidmann L, Kamyschanskiy Y, Wagner DC, Zimmer S, Roth W. CD15 immunostaining improves placental diagnosis of fetal hypoxia. *Placenta* 2021;105:41–9.
90. Mitlid-Mork B, Turowski G, Bowe S, Staff AC, Sugulle M. Circulating angiogenic profiles and histo-morphological placental characteristics of uncomplicated post-date pregnancies. *Placenta* 2021;109:55–63.
91. R Core Team. R. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015. Available from: <https://www.R-project.org/>.
92. Castellucci M, Scheper M, Scheffen I, Celona A, Kaufmann P. The development of the human placental villous tree. *Anat Embryol* 1990;181:117–28.
93. Kaufmann P, Mayhew TM, Charnock-Jones DS. Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. *Placenta* 2004;25:114–26.
94. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000;6:389–95.
95. Charnock-Jones DS, Kaufmann P, Mayhew TM. Aspects of human fetoplacental vasculogenesis and angiogenesis. I. Molecular regulation. *Placenta* 2004;25:103–13.
96. Demir R, Kaufmann P, Castellucci M, Erbengi T, Kotowski A. Fetal vasculogenesis and angiogenesis in human placental villi. *Acta Anat* 1989;136:190–203.
97. Demir R, Seval Y, Huppertz B. Vasculogenesis and angiogenesis in the early human placenta. *Acta Histochem* 2007;109:257–65.
98. 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.
99. Mayhew TM, Charnock-Jones DS, Kaufmann P. Aspects of human fetoplacental vasculogenesis and angiogenesis. III. Changes in complicated pregnancies. *Placenta* 2004;25:127–39.
100. Pinar H. *The Human placenta: normal developmental biology*. Warwick, Rhode Island: Core Curriculum Publishers, LLC; 2009.
101. Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. *Reproduction* 2009;138:895–902.
102. Ahmed A, Dunk C, Ahmad S, Khaliq A. Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) and soluble Flt-1 by oxygen – a review. *Placenta* 2000;21(A Suppl):S16–24.
103. Jirkovská M, Janáček J, Kaláb J, Kubínová L. Three-dimensional arrangement of the capillary bed and its relationship to microrheology in the terminal villi of normal term placenta. *Placenta* 2008;29:892–7.
104. Oosterhof H, Wichers G, Fidler V, Aarnoudse JG. Blood viscosity and uterine artery flow velocity waveforms in pregnancy: a longitudinal study. *Placenta* 1993;14:555–61.
105. Steel SA, Pearce JM, Nash G, Christopher B, Dormandy J, Bland JM. Maternal blood viscosity and uteroplacental blood flow velocity waveforms in normal and complicated pregnancies. *Br J Obstet Gynaecol* 1988;95:747–52.
106. Benirschke KKP, Baergen RN *Pathology of the human placenta*, 5th ed. New York: Springer-Verlag New York; 2006, vol XX:1050 p.
107. Castellucci M, Kaufmann P. A three-dimensional study of the normal human placental villous core: II. Stromal architecture. *Placenta* 1982;3:269–85.
108. 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.
109. Daskalakis G, Marinopoulos S, Krieles V, Papapanagiotou A, Papantoniou N, Mesogitis S, et al. Placental pathology in women with gestational diabetes. *Acta Obstet Gynecol Scand* 2008;87: 403–7.
110. 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.
111. Christians JK, Gynspan D. Placental villous hypermaturation is associated with improved neonatal outcomes. *Placenta* 2019;76: 1–5.
112. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, et al. The preterm parturition syndrome. *BJOG* 2006;113:17–42.
113. Pearce P, Brownbill P, Janacek J, Jirkovska M, Kubinova L, Chernyavsky IL, et al. Image-based modeling of blood flow and oxygen transfer in feto-placental capillaries. *PLoS One* 2016;11: e0165369.
114. Schneider H. Ontogenic changes in the nutritive function of the placenta. *Placenta* 1996;17:15–26.
115. Mayhew TM, Simpson RA. Quantitative evidence for the spatial dispersal of trophoblast nuclei in human placental villi during gestation. *Placenta* 1994;15:837–44.
116. Schoots MH, Gordijn SJ, Scherjon SA, van Goor H, Hillebrands JL. Oxidative stress in placental pathology. *Placenta* 2018;69:153–61.
117. Stanek J. Hypoxic patterns of placental injury: a review. *Arch Pathol Lab Med* 2013;137:706–20.