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Trophoblast proliferation is higher in female than in male preeclamptic placentas☆

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ABSTRACT

Introduction: Preeclampsia (PE) is a pregnancy-specific hypertensive disorder with inflammatory complications. There are no known placental histopathological features, which are unique to PE. It is often pooled with the fetal growth restriction (FGR) under a single umbrella pathophysiology, the maternal vascular malperfusion (MVM). The aim of this study is to assess the villous trophoblast and the villous tree quantitatively in PE placentas and to identify morpholgical correlates unique to PE.

Methods: 20 PE placentas (10 female and 10 male) and 20 Control placentas (10 female and 10 male) were included in the study. The villous trophoblast and the villous tree were assessed quantitatively by Stereology and 3D Microscopy. For Stereology measurements, the villous tree was classified in contractile and non-contractile parts based on immunohistochemical detection of perivascular myofibroblasts.

Results: The density of proliferative trophoblast nuclei is increased, whereas the density of non-proliferative trophoblast nuclei is decreased in female PE placentas. The male PE placentas do not show this effect. Though no significant difference in the diffusion distance was observed, the non-contractile villi and the fetal vessels inside show a significantly reduced volume in PE placentas. The branching index of the villous tree is lower in PE placentas in general. However, in female PE placentas the deviation is accentuated.

Conclusion: In PE, the villous trophoblast shows a sexually dimorphic alteration in the density of proliferative and non-proliferative nuclei, which is inherently different from FGR.

1. Introduction

Preeclampsia (PE), a pregnancy-specific hypertensive and pro-inflammatory disorder, affects 2–8% of pregnancies worldwide, and is a significant cause of maternal and fetal morbidity and mortality [1]. PE requires the placenta to evolve, but does not require the presence of a conceptus [2] or an endometrial implantation site [3]. The only curative treatment for preeclampsia is delivery. These observations underline the central role of the placenta in PE. Fetal growth restriction (FGR) and PE are thought to be related by a common underlying phenomenon called maternal vascular malperfusion [4]. The relation of both syndromes is also reflected by their designation as ischaemic placental disease [5,6]. The post-partum histopathological examination of the placentas of PE pregnancies involves the assessment of various features, including Tenney-Parker changes, villous maturity, infarctions, villitis of unknown etiology, increased fibrinoid deposition, and atherosis [7,8]. However, none of these findings is unique to PE; they can also be associated with conditions such as FGR or, though less commonly, occur in normal placentas [8]. This is limiting the clinical value of post-partum histopathologic examination of PE-placentas for obstetricians. Still, the syndrome can only be diagnosed clinically. Moreover, while sexual dimorphism in e.g. cytokine production, apoptosis [9], lipid metabolism [10] and hormone levels [11] has been described in PE, histopathological correlates of these fetal sex-related clinical observations are

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currently beyond the scope of routine post-partum histopathology. PE as an obstetric syndrome is characterised by various clinical issues, with the most critical being hypertensive and inflammatory complications [12,13] in the mother, although fetal growth can also be affected. Moreover, PE doubles the risk of lifetime cardiovascular disease for the mother [14]. The placental tissue that plays a key role in releasing pro-inflammatory signals into the maternal bloodstream is the villous trophoblast, which is an epithelial layer situated at the junction of the maternal (uteroplacental) and fetal (fetoplacental) circulations [15,16]. Material shed or released from this region enters the maternal bloodstream, exits the placenta, reaches the lungs, and can potentially impact the entire maternal body. In cases of PE, there is an elevated amount of shedding from the villous trophoblast into the uteroplacental circulation [16]. A significant association has been found between plasma miR-125b levels and PE [17]. Analysis of DNA methylation in cell-free fetal DNA (cfDNA) found in the mother's plasma during PE has shown changes in amount [18] and in methylation patterns, which might even have predictive value for severe early onset PE [19] or uncategorised PE [20,21]. This indicates that there could be qualitative alterations in the nature and composition of the materials being shed. Recently, we were able to demonstrate by means of advanced 3D-microscopy and stereology structural sexual dimorphism and morphological alterations associated with the maturation of human villous trophoblast in cases of fetal growth restriction [22,23]. The current study has been designed similarly and is analysing the proliferation and maturation of the villous trophoblast and the villous tree in PE. It identifies morphologic features unique to PE and morphological correlates of placental sexual dimorphism in PE.

2. Materials and methods

All methods and procedures were conducted in compliance with relevant guidelines and regulations, for which the ethics board of Ludwig-Maximilians-University of Munich (LMU Munich) has granted approval under the numbers 084–11 and 478–12.

2.1. Clinical groups and placental tissue

20 term placentas from clinically non-pathological pregnancies (Control, 10♀, 10♂) and 20 placentas from pregnancies with pre-eclampsia (PE, 10♀, 10♂) were analysed for this study. PE was clinically diagnosed according to the guidelines as new-onset hypertension with a systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg and the existence of proteinuria (≥300 mg/d) or other end-organ damage [24]. A co-occurrence of FGR or gestational diabetes mellitus (GDM) was an exclusion criterion. The scope of the ethical vote did not encompass any maternal confounding factors like BMI, smoking etc. All placentas were obtained from the Department of Obstetrics and Gynaecology at the "Dritter Orden" hospital in Munich, Germany. The placentas were collected only after obtaining informed consent from the mothers/parents. The groups were matched based on the mode of delivery (caesarean section only). The matching of gestational age is not possible, as any placenta born before term cannot be considered as control, even when there is no known pathology associated with it. Essential clinical data and gross anatomical placental information are presented in Table 1.

Immediately after birth, all placentas were cooled at a temperature of 4 °C and transported under continuous refrigeration to the Department of Anatomy II at LMU Munich. At the department, weight, thickness and diameters of the placentas were measured, before taking whole-depth samples. Tissue sampling was carried out using a well-established systematic and random procedure [22]. With this procedure, six slides per placenta were analysed by stereology and one sample of the villous tree per placenta was analysed by 3D Microscopy.

Table 1
Mean values and respective standard errors in parentheses for routine clinical and macroscopic parameters are given in the table for (top) Stereology and (bottom) 3D Microscopy. Statistically significant differences, which are independent of sex, are indicated by A. Further, * denotes a significant difference from the control value for a specific group. The birthweight percentile values are based on [44].

	Stereology			
Variable	Control		PE	
	male (n = 10)	female (n = 10)	male (n = 10)	female (n = 10)
Gestational Age (week)[A]	38.8 (0.4)	39.3 (0.2)	34.7 (1.2)*	35.0 (1.5)
Birth Weight (g)[A]	3557 (145)	3408 (177)	2279 (287)*	2330 (426)
Birth Weight (Percentile)[A]	49 (9)	46 (11)	21 (6)	31 (10)
Placental Weight (g)[A]	690 (115)	574 (38)	396 (40)*	398 (62)
Ratio PW/BW	0.19 (0.02)	0.17 (0.01)	0.20 (0.02)	0.18 (0.01)
Surface Area (cm^2)[A]	1250 (60)	1127 (66)	967 (119)*	907 (90)
Longest Diameter (cm)[A]	21.1 (0.6)	20.1 (0.6)	19.1 (1.4)	18.5(0.8)
Shortest Diameter (cm)[A]	18.8 (0.4)	17.8 (0.7)	15.7 (0.8)*	15.4 (0.9)
Thickness (cm)	1.8 (0.1)	1.6 (0.1)	1.4 (0.1)	1.3 (0.2)
Roundness	1.13 (0.03)	1.14 (0.04)	1.21 (0.06)	1.23 (0.07)
	3D Microscopy			
Variable	Control		PE	
	male (n = 10)	female (n = 10)	male (n = 3)	female (n = 6)
Gestational Age (week)	38.8 (0.4)	39.3 (0.2)	36.9 (0.9)	34.7 (2.3)
Birth Weight (g)	3557 (145)	3408 (177)	2853 (284)	2432 (629)
Birth Weight (Percentile)	49 (9)	46 (11)	28 (12)	35 (14)
Placental Weight (g)	690 (115)	574 (38)	466 (28)	414 (101)
Ratio PW/BW	0.19 (0.02)	0.17 (0.01)	0.17 (0.02)	0.18 (0.01)
Surface Area (cm^2)[A]	1250 (60)	1127 (66)	947 (7)*	927 (135)
Longest Diameter (cm)[A]	21.1 (0.6)	20.1 (0.6)	18.7 (0.3)*	18.7(1.2)
Shortest Diameter (cm)[A]	18.8 (0.4)	17.8 (0.7)	16.2 (0.2)*	15.5 (1.4)
Thickness (cm)	1.8 (0.1)	1.6 (0.1)	1.8 (0.1)	1.4 (0.3)
Roundness	1.13 (0.03)	1.14 (0.04)	1.16 (0.03)	1.24 (0.11)

2.2. 3D microscopy

All control and a subset of 12 PE placentas (9♀, 3♂) were measured by 3D Microscopy by a single operator. For the measurements, small bushes of the peripheral villous tree were isolated and processed as described in detail in Ref. [22] and in Supplementary Table 1. Briefly, the villous trees were first fixed in 4.5 % formaldehyde. The proliferative nuclei were stained selectively by immunohistochemical reaction targeting proliferating cell nuclear antigen (PCNA). Non-proliferative nuclei were counterstained using hematoxylin. The isolated villous tree was then transferred on a concave slide to achieve a whole-mount preparation for tracing. Manual tracing of all peripheral villous trees was performed using Neurolucida software (version 11.02; MBF Bioscience, Williston, VT, USA) under a brightfield microscope with a 20× objective. The tracing of at least two outermost branch generations was done in the direction from the proximal end to the terminal end of the peripheral villous tree. The quantified data encompassed branching angles, villous diameters, lengths, villous surface area and villous volume. Also, the number of PCNA positive and PCNA negative nuclei,

together with their individual 3D coordinates, were registered. The data were stratified according to the branch generation.

2.3. Stereology

Representative thin sections were taken from the whole-depth placental blocks and quantified by stereology as described in Ref. [25]. The immunohistochemical double staining of the perivascular sheath and the fetal villous endothelium was performed by targeting γ-sm-actin and CD34 respectively. The staining procedure is described in detail in Supplementary Table 2. Villous cross-sections showing the presence of myofibroblasts through perivascular labeling of γ-sm-actin were categorized as contractile villi (C-villi), while those without myofibroblasts were classified as non-contractile villi (NC-villi) (Fig. 1A–B). Counting points falling within an intravillous vascular lumen were registered as a partial volume of the villous volume and inherited the property of belonging to either C-villi or NC-villi. With the "Nearest Neighbour Workflow" of Stereo Investigator (MBF Bioscience, Williston, USA) volume fractions of γ-sm-actin positive and γsm-actin negative components of the villous trees, the volume of the intervillous space and the diffusion distance were estimated. Further, the branching index was determined as described in Ref. [23] by building a ratio of the counting

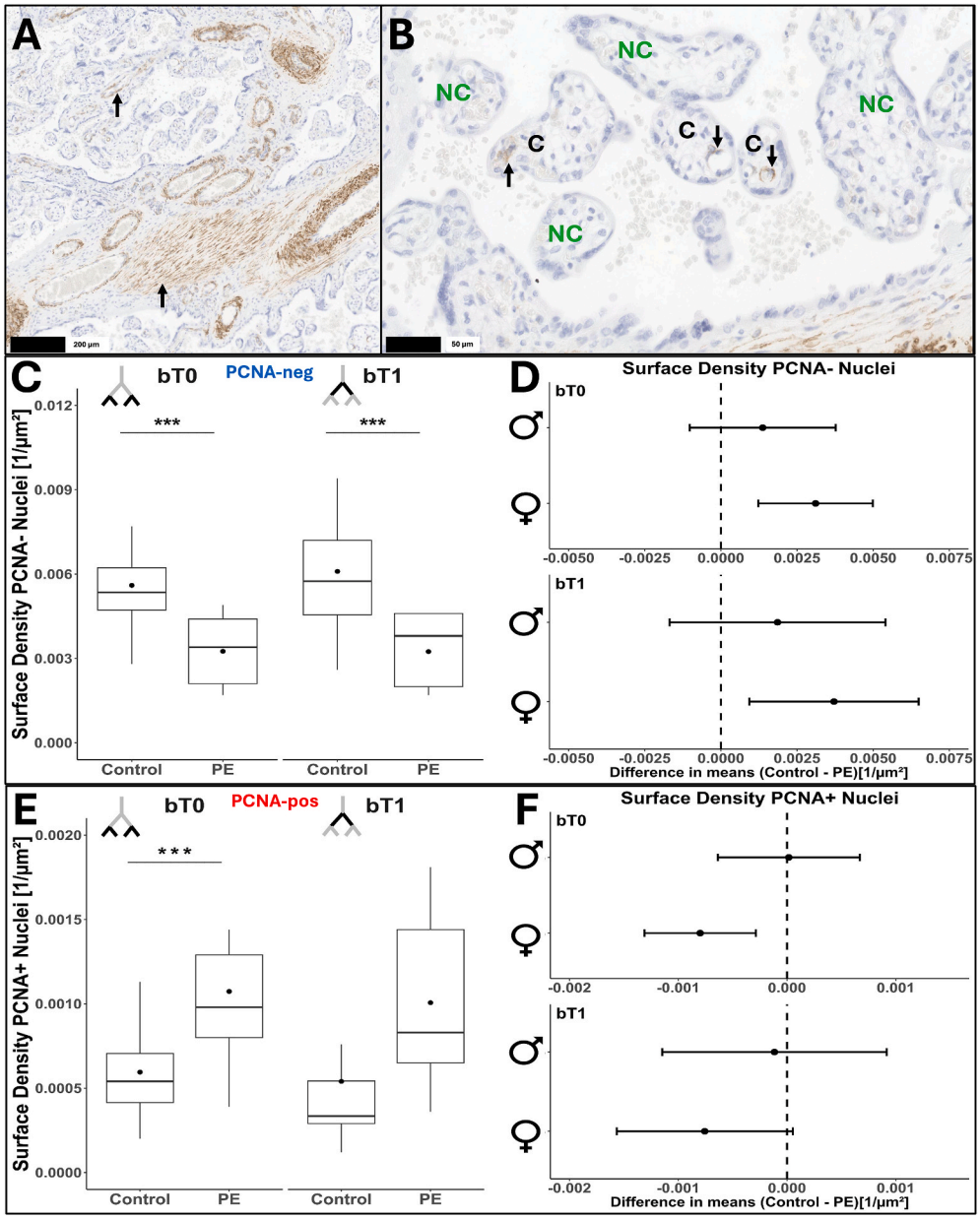

Fig. 1. Tiles A and B show immunohistochemical detection of γ-sm-actin in sections of formalin fixed and paraffin-embedded placental tissue. Bars represent 200 µm in A, and 50 µm in B. A: Arrows point to γ-sm-actin positive perivascular regions in high-caliber and mid-caliber villi. B: Various small caliber villi with perivascular γ-sm-actin (arrows; villi labelled as contractile villi (C)). Villi without perivascular γ-sm-actin are labelled as non-contractile villi (NC). Tiles C to F show the mean surface density of cell nuclei of villous trophoblast for either PCNA-negative (C, D) or PCNA-positive cell nuclei (E,F) in terminal (bT0) or pre-terminal (bT1) branches of the villous tree. For group comparisons statistical significance is indicated above the Tukey plots by asterisks ($p < 0.001$(***)) C: The Tukey plots show significantly reduced density of PCNA-negative cell nuclei of villous trophoblast of PE placentas in bT0 and bT1 branches. The Forest plots in D demonstrate that the difference between groups shown in C is driven by female placentas, not by male placentas. E: The Tukey plots show significantly increased density of PCNA-positive cell nuclei of villous trophoblast of PE placentas in bT0, but not in bT1 branches. The Forest plots in F demonstrate that the difference between groups shown in E is driven by female placentas, not by male placentas.

points falling on concave and convex villous surfaces. All stereological procedures were performed by a single operator.

2.4. Statistical analysis

The software R [26] was used to calculate the mean and standard deviation for all the parameters investigated. Comparisons and statistical testing between the groups were performed using robust statistical methods on trimmed, Winsorized means according to Yuen [27,28]. The R package "ggstatsplot" was used for statistical testing and visualisation of graphs [29].

3. Results

3.1. Villous trophoblast

Mean Surface Density (MD) of Cell Nuclei in Villous Trophoblasts: The MD of PCNA-negative nuclei on the surface of villous trophoblast for both, terminal (bT0) and preterminal (bT1), branches within the pre-eclampsia (PE) group was significantly reduced compared to the Control group ($p < 0.01$, Fig. 1C). This variance is predominantly attributable to the female villous trophoblast (Fig. 1D). The MD of PCNA-negative nuclei in the male villous trophoblast did not show any significant difference (Fig. 1D).

The MD of PCNA-positive cell nuclei on the surface of bT0 branches, but not bT1 branches, in the PE group was significantly elevated, when compared to the Control group (bT0: $p < 0.01$, Fig. 1E). This distinction is exclusively due to female villous trophoblast (Fig. 1F). Within bT1 branches, the MD exhibited a non-significant inclination towards the same pattern observed in bT0 branches, also influenced solely by female villous trophoblast (Fig. 1F).

Mean Nearest Neighbour Distance (NND): The NND for PCNA-negative nuclei of villous trophoblast, unlike that for PCNA-positive nuclei of villous trophoblast, was significantly greater in PE placentas than in Control placentas ($p < 0.01$, Fig. 2A–B).

Diffusion Distance: No significant difference was observed in the diffusion distance and the standard deviation of the diffusion distance between Control and PE groups (Table 2).

Sexual Dimorphism: In the Control group, the NND for PCNA-negative nuclei of villous trophoblast was significantly greater in male placentas than in female placentas (Fig. 2C). This pattern of sexual dimorphism was absent in the PE placentas (Fig. 2C).

Table 2
Mean values and respective standard errors for a selection of estimates, as determined by Stereology, are given in the table. Statistically significant differences are indicated with p-values greater than 0.05 as not significant (ns), whereas p-values less than 0.05 are indicated as (*).

Variable		Control (n = 20)	PE (n = 20)	p-Value
		10 male, 10 female	10 male, 10 female	
Total Villous Volume (ml)		290.9 ± 26.7	188.6 ± 28.8	*
Volume IVS (ml)	male	380.6 ± 67.5	227.2 ± 19.6	ns
	female	302.0 ± 17.8	189.8 ± 29.9	*
Ratio Contractile/Non-contractile		0.7 ± 0.0	1.1 ± 0.2	ns
Volume Villi (ml)	male	120.1 ± 20.8	72.2 ± 10.0	*
	female	110.5 ± 10.5	98.6 ± 26.9	ns
Volume Capillaries (ml)	male	27.9 ± 5.06	21.5 ± 3.73	ns
	female	29.0 ± 4.12	32.3 ± 10.2	ns
Branching Index of Capillaries	male	3.88 ± 0.48	4.19 ± 0.75	ns
	female	4.67 ± 0.68	3.17 ± 0.31	ns
Diffusion distance (μm)		18.4 ± 0.7	16.6 ± 0.7	ns
Volume Villi (ml)	male	189.4 ± 28.6	96.6 ± 14.1	*
	female	161.8 ± 16.1	109.5 ± 28.2	*
Volume Capillaries (ml)	male	163.6 ± 25.0	83.4 ± 11.8	*
	female	137.9 ± 13.5	94.1 ± 23.2	*
Branching Index of Capillaries	male	5.19 ± 0.39	5.26 ± 0.48	ns
	female	6.13 ± 0.61	4.80 ± 0.61	*
Diffusion distance (μm)		8.7 ± 0.3	8.1 ± 0.4	ns

3.2. Villous tree

Villous Volume: The total villous volume in female PE placentas does not show a significant decrease compared to female Control placentas, although it trends towards being smaller ($p = 0.051$). Conversely, the total villous volume in male PE placentas is significantly reduced in comparison to male Control placentas ($p < 0.01$, Fig. 3A). There is no significant difference in the absolute volume of C-villi between PE and Control placentas (Fig. 3B). However, when differentiated according to sex, in male PE placentas, the absolute volume of C-villi is significantly less ($p < 0.05$) than in male Control placentas (Table 2). The absolute volume of NC-villi in PE placentas is significantly reduced compared to the Control group (Fig. 3B). The decrease occurs in both, male and female placentas (males: $p < 0.05$; females: $p < 0.05$) (Table 2). The proportion of C-villi to NC-villi (C-villi/NC-villi ratio) does not show a significant variance between the PE and Control placentas of either sex

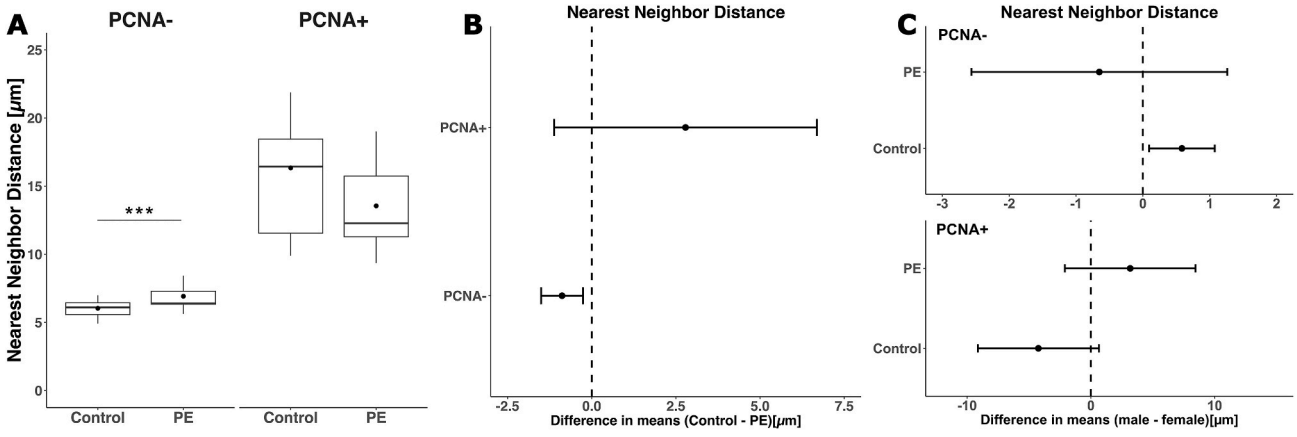

Fig. 2. A–C: Nearest neighbour distances (NND) of cell nuclei of villous trophoblast. Tukey plots in A show the group comparisons of NND of PCNA-negative cell nuclei (significantly longer in PE ($p < 0.001$(***)) and PCNA-positive cell nuclei (not significantly different). B: The Forest plots correspond to the data of A and illustrate the smaller confidence intervals associated with NND of PCNA-negative cell nuclei than with NND of PCNA positive cell nuclei. C: The Forest plots are based on the comparison of sexes (male - female) of NND of PCNA-negative and PCNA-positive cell nuclei of villous trophoblast. In the Control group, the NND of PCNA-negative cell nuclei of female villous trophoblast is significantly shorter than the NND of male villous trophoblast.

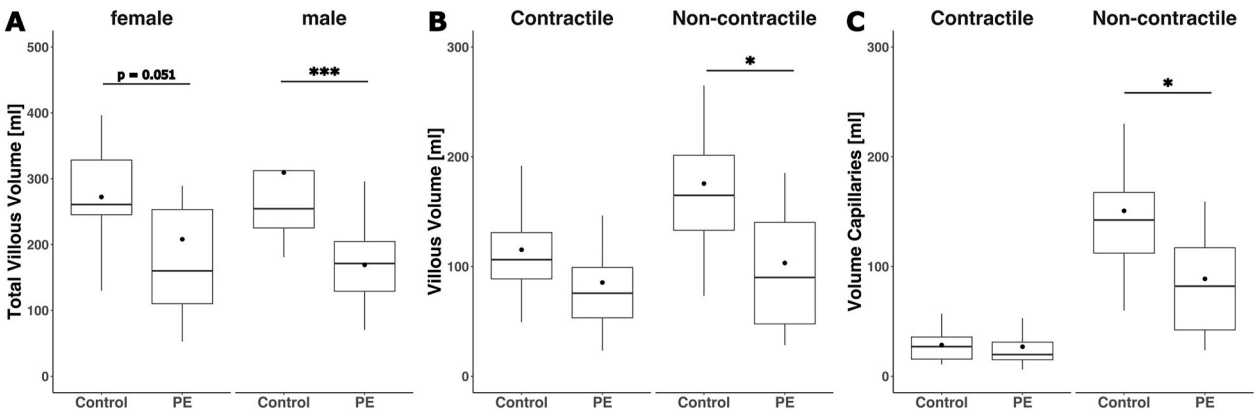

Fig. 3. Tukey plots in A show group comparisons of the total villous volumes categorized by gender. Tukey plots B show group comparisons of villous volume classified by its contractility based on the immunohistochemical detection of perivascular γ-smooth-muscle actin. The volume of the fetal capillaries within each villous type is compared between the groups in C. Statistical significance is visually indicated with p-values greater than 0.05 denoted as not significant (ns), p-values less than 0.05 as (*), p-values less than 0.01 as (**) and p-values less than 0.001 as (***).

(Table 2).

Furthermore, the intravascular volume within C-villi remains consistent between male and female PE and Control placentas (Fig. 3C and Table 2). However, for both male and female placentas, the absolute vessel volume within NC-villi in PE placentas is significantly less than in Control placentas (males: $p < 0.05$; females: $p < 0.05$, Fig. 3C and Table 2).

The volume of the intervillous space in female PE placentas is significantly diminished relative to female Control placentas ($p < 0.05$), a contrast not seen in male placentas (Table 2).

Villous Branching: Neither the planar branching angles nor the tortuosity of villous tree branches showed any significant difference between the groups. However, in both C-villi (males: $p < 0.01$; females: $p < 0.001$) and NC-villi (males: $p < 0.05$; females: $p < 0.001$), the branching index in both female and male PE placentas is significantly lower than in their female and male Control counterparts (Fig. 4). This reduction is observed across both sexes, with a slightly more marked effect in female placentas.

There are no notable differences in the branching index of capillaries within the C-villi of PE placentas when compared to Control placentas for both sexes (Table 2). The branching index of capillaries in NC-villi of female PE placentas is significantly reduced ($p < 0.05$) compared to that in female Control placentas (Table 2).

4. Clinical and macroscopic parameters

The routine clinical and placental macroscopic parameters for both groups are listed in Table 1. For male newborns from PE pregnancies, both the birth weight (BW) and placental weight (PW), along with the gestational age (GA), were significantly lower (BW: $p < 0.001$; PW: $p < 0.05$; GA: $p < 0.001$) in comparison to those of male newborns from Control pregnancies. The variation in BW and GA was more pronounced amongst female than male newborns. When considering newborns of either sex, BW, PW, and GA were notably smaller in PE pregnancies (BW: $p < 0.001$; PW: $p < 0.001$; GA: $p < 0.001$) compared to those from Control pregnancies (Table 1).

The surface area (SA) and the smallest diameter (SD) of male placentas from PE pregnancies were significantly reduced (SA: $p < 0.05$; SD: $p < 0.01$) when compared to those in the male control group. Across sexes, the SA, SD, and the longest diameter (LD) in PE placentas were significantly lesser (SA: $p < 0.01$; SD: $p < 0.001$; LD: $p < 0.05$) than those in Control placentas (Table 1).

There were no significant differences observed in any clinical or macroscopic parameters between the PE and Control groups for female newborns/placentas. Moreover, no significant differences were noted in the thickness or roundness of placentas between the PE and Control groups, irrespective of the newborn's sex (Table 1).

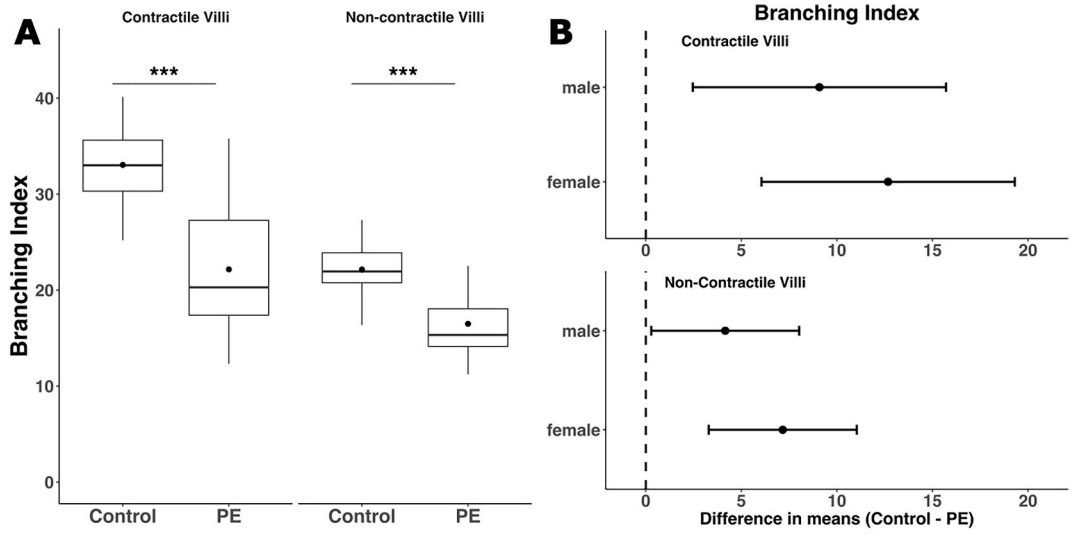

Fig. 4. A–B: The Tukey plots in A demonstrate that the Branching Index of contractile villi and non-contractile villi is significantly reduced in PE ($p < 0.001$(***)). The Forest plots in B show the data of A by gender and show that the significant difference between Controls and PE can be found independent of sex.

5. Discussion

The present study's data indicate that there is sexual dimorphism in the villous trophoblast located at the feto-maternal interface of the villous tree, as well as sexually monomorphic changes in the branching patterns of the villous tree in placentas affected by preeclampsia (PE).

5.1. Villous trophoblast

Sexually dimorphic spatial arrangements of PCNA-negative villous trophoblast nuclei are evident in the current study, even in normal (Control) placentas. These PCNA-negative nuclei belong to the post-proliferative fraction of villous trophoblast [30]. In Control placentas, the distances between nuclei in male villous trophoblast are greater than in female villous trophoblast, and the density of male nuclei at the surface is lower than the density of female nuclei (Fig. 1). This sexually dimorphic arrangement of nuclei at the villous surface is already known in normal placentas from a related study on fetal growth restriction (FGR) [22]. Therefore, the data from the current study support the idea that this type of sexual dimorphism is a fundamental characteristic of healthy villous trophoblast in Control placentas.

However, this Control-related sexual dimorphism at the villous surface is not observed in the PE placentas examined in this study. Instead, there is a specific and sexually dimorphic alteration of the Control state at the villous surface, indicating disruption of trophoblast structure and possibly function. The increased concentration of PCNA-positive nuclei, along with a decreased concentration of PCNA-negative nuclei, observed in the entire PE group, is primarily driven by the female PE placentas rather than the male PE placentas. The male PE placentas do not show statistically significant effects of PE on the density of PCNA-positive or PCNA-negative nuclei.

This indicates a unique type of sexual dimorphism specific to PE in villous trophoblast, which is easily distinguishable from the sexual dimorphism observed in normal placentas. Additionally, this differs from the findings in cases of FGR, where there is no sexual dimorphism of villous trophoblast observed in the affected placentas. Furthermore, the change in density of trophoblast nuclei in FGR placentas is qualitatively different from the change observed in PE placentas [22].

Sexual dimorphism in PE has been observed in previous studies, primarily at the biochemical or molecular biological level [9,10,31], and occasionally at the epidemiological level [32,33]. The discovery of distinct sexual dimorphisms at the structural microscopic level in the trophoblast of PE placentas, as demonstrated in this study, adds a new dimension to our understanding of sexual dimorphism in human trophoblast. However, at this stage of research, the question of how the biochemical and epidemiological aspects relate to the morphological dimorphism of human trophoblast remains unanswered.

The results of the current study on villous trophoblast in PE can be functionally interpreted as an increase in the amount of trophoblast material being processed and a faster passage through the syncytium (Fig. 5). This perspective suggests that the higher concentration of PCNA-positive trophoblast nuclei indicates a greater assimilation of trophoblast nuclei into the syncytium over time. The lower density of PCNA-negative nuclei is seen as an indication of an accelerated passage through the syncytial stage of villous trophoblast. Ultimately, both mechanisms contribute to a higher release of trophoblast materials from the trophoblast surface into the maternal circulation (Fig. 5). Excess syncytiotrophoblast shedding has been shown as a distinct feature of PE [16]. Additionally, the faster passage through the syncytial stage of villous trophoblast may increase the proportion of prematurely shed material from the villous surface. This could suggest that the increased amounts of shed materials have a different composition compared to shed materials in Control placentas. A drastic increase in cell-free fetal DNA, which can be interpreted as a consequence of increased trophoblast shedding, has been observed in PE cases [18,20,21] and was found to be associated with changes in the methylome [19]. Such prematurely released material may have a higher pro-inflammatory potential than trophoblast material released after sufficient maturation. These changes could potentially be a significant factor in driving the pro-inflammatory status in mothers with PE. That mothers of female fetuses are more vulnerable in case of PE is supported by epidemiological data [32,34], which show that PE mothers with female fetuses are the majority of those with preterm delivery. Not before term there is a normalisation of male/female ratio to about 1.

5.2. Branching patterns of the villous tree

The present study is providing evidence of a major difference in the branching pattern of the villous tree – irrespective of fetal sex. The reduction in branching affects the whole villous tree, the central

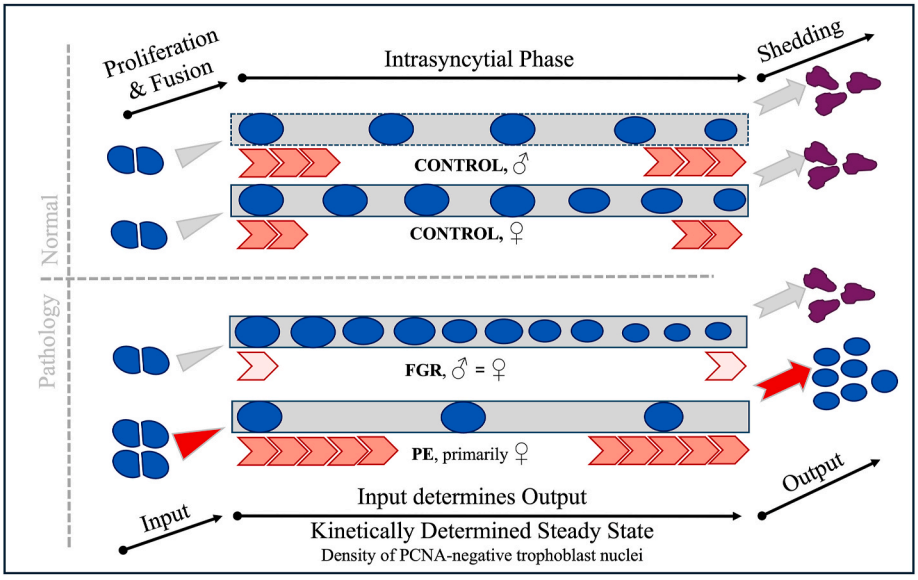

Fig. 5. The figure shows a schematic representation of the passage of trophoblast nuclei in control (top) and pathologic cases (bottom). The blue circles represent the trophoblast nuclei, which proliferate (left) and fuse with the syncytium (center) before being shed (right) in the maternal circulation. The red arrows indicate the speed of transition through the intrasyncytial phase.

contractile parts as well as the peripheral non-contractile exchange areas. Though the difference is significant for both the sexes, the female placentas show a more pronounced effect. We are not aware of any published quantitative estimates of the villous branching in PE. Nevertheless, the hypothesis of increased villous branching as a compensatory mechanism in PE-related hypoxic conditions [35,36] cannot be supported by our finding.

Minor effects were seen on the volumes of the whole villous trees. The volume of NC-villi and the volume of fetal capillaries inside the NC-villi are reduced in PE placentas. In particular, the reduction in the volume of NC-villi in PE is a significant departure from what is observed in FGR, where no such reduction was observed [23]. A previous quantitative study, employing similar methods as described in our study, found no significant difference in the villous volume of PE placentas [37]. In this study the components of the villous tree were classified as stem, intermediate and terminal villi. It has been shown that this classification is subjective and prone to rater bias [38]. To remove any rater bias we classify the villous tree objectively based on the presence of perivascular myofibroblast. Therefore, despite the closeness in the approaches, the results of these studies cannot be compared against one another.

5.3. Clinical and macroscopic data

The findings of the current study demonstrate that in the PE group, regardless of fetal sex, there is a significant decrease in birth weight, placental weight, and gestational age. These findings are consistent with previous research [39–41]. However, it is likely that the relationship between lower birth weight and placental weight and the development of preeclampsia is not as strong as indicated by our study data [39]. The reduced gestational age in the PE group directly affects both placental weight and birth weight. In cases of preeclampsia, indications for late preterm delivery are often related to maternal or iatrogenic factors and may not necessarily indicate placental dysfunction [39,42]. Premature delivery itself is associated with lower birth weight and placental weight.

There is an ongoing discussion in the field regarding the extent to which preeclampsia alone is capable of significantly altering birth weight and placental weight [43]. Further research is needed to fully understand the complex relationship between preeclampsia, placental weight, and birth weight.

5.4. Strengths and limitations

This study quantifies the histomorphological parameters of the villous trophoblast in PE placentas and shows that the excessive shedding of syncytiotrophoblast is probably related to increased trophoblast proliferation, especially in female PE placentas. The primary aim of this study was to investigate PE placentas that do not show co-occurrence of neither FGR nor GDM. Any other maternal confounding factors like BMI, smoking etc. have not been considered.

The sample size of male PE placentas is lower than the female PE placentas. Though robust statistical methods were applied for statistical testing, which are relatively insensitive to unequal sample sizes, unequal variances and non-normal distributions, the possibility of an insufficient statistical power in case of male PE placentas can not be ruled out.

6. Conclusion

The altered density of proliferative and non-proliferative trophoblast nuclei is so far the only histopathologic feature that is unique to PE.

CRediT authorship contribution statement

N. Barapatre: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **L. Hansen:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **C. Kampfer:** Writing – review & editing, Formal analysis. **T. Rübelmann:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **C. Schmitz:** Writing – review & editing, Supervision, Resources. **F. von Koch:** Writing – review & editing, Supervision, Resources, Conceptualization. **H.G. Frank:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2024.10.016.

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