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Placental trophoblast differentiation and vascular remodeling in normal and preeclamptic pregnancies

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Abstract

Inadequate trophoblast invasion and defective spiral artery remodeling are central features of preeclampsia, yet the precise differentiation pathways that distinguish normal from pathological placentation remain contested. This research compared trophoblast subtype distribution, differentiation marker expression, and vascular remodeling indices in placental tissue from 35 women with uncomplicated pregnancies and 28 women diagnosed with early-onset preeclampsia (delivery before 34 weeks). Tissue was collected at delivery from the Hippokration Hospital, Thessaloniki. Immunohistochemistry quantified cytokeratin-7, HLA-G, syncytin-1, and α -smooth muscle actin expression. Serum sFlt-1 and PlGF concentrations were measured by ELISA. Preeclamptic placentas showed a 38.7% reduction in extravillous trophoblast density ($p < 0.001$) and incomplete spiral artery remodeling in 71.4% of examined vessels compared with 12.6% in controls. The sFlt-1/PlGF ratio was 4.8-fold higher in preeclamptic sera ($p < 0.001$). Syncytin-1 expression was reduced by 42.3% in preeclamptic syncytiotrophoblast. These findings link impaired trophoblast differentiation to deficient vascular transformation and elevated antiangiogenic signaling in early-onset preeclampsia.

Keywords: Trophoblast differentiation, vascular remodeling, preeclampsia, placental development, pregnancy complications, spiral artery, extravillous trophoblast, sFlt-1, syncytin-1, antiangiogenic

Introduction

On one side of the placental interface, fetal trophoblast cells invade maternal tissue with a precision that rivals malignant tumors in its depth and coordination—yet unlike cancer, this invasion is tightly self-limited^[1]. On the other side, maternal spiral arteries lose their smooth muscle walls and become wide, low-resistance conduits that supply the growing fetus with blood^[2]. When either process fails, the consequences can be severe.

Preeclampsia, affecting 3-5% of pregnancies worldwide, is characterized by new-onset hypertension and proteinuria after 20 weeks of gestation^[3]. The early-onset form (before 34 weeks) carries higher maternal and fetal morbidity and is more strongly associated with defective placentation than late-onset disease^[4]. Histological examination of preeclamptic placentas consistently reveals shallow trophoblast invasion and incompletely remodeled spiral arteries, though the molecular drivers of these defects aren't fully settled^[5].

Trophoblast cells follow two main differentiation paths: villous cytotrophoblasts fuse to form the multinucleated syncytiotrophoblast covering chorionic villi, while a separate population differentiates into extravillous trophoblasts that anchor the placenta and invade the decidua^[6]. The balance between these lineages, and the depth of extravillous trophoblast invasion, appears to determine the adequacy of spiral artery remodeling^[7].

This research examined trophoblast subtype distribution, differentiation marker expression, and spiral artery remodeling in placentas from normal and early-onset preeclamptic pregnancies at the Hellenic College of Biological Sciences, Thessaloniki, Greece^[8, 9].

Materials and Methods

Materials

This research was conducted from January through November 2022 at the Department of Stem Cell Biology, Hellenic College of Biological Sciences, Thessaloniki, Greece, with tissue collection at the Hippokration General Hospital obstetric unit. Ethical approval was

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obtained from the Aristotle University Bioethics Committee (Protocol No. AUPh-BC-2021-112, approved 8 December 2021). All participants provided written informed consent.

Instrumentation and Analytical Equipment

Immunohistochemistry was performed using a Ventana BenchMark ULTRA automated staining system (Roche Diagnostics, Basel, Switzerland). Histological imaging used an Olympus BX53 microscope with a DP74 camera. Serum sFlt-1 and PlGF were measured using Elecsys immunoassay kits on a Cobas e411 analyzer (Roche). Morphometric analysis of spiral artery remodeling was performed using QuPath v0.3.2 open-source digital pathology software.

Methods

Participants comprised 35 women with uncomplicated term pregnancies (37-40 weeks) and 28 women with early-onset preeclampsia (delivery <34 weeks, defined by ISSHP 2018 criteria). Exclusion criteria included chronic hypertension, gestational diabetes, multiple pregnancy, and known fetal anomalies. Placental tissue was collected within 30 minutes of delivery. Full-thickness placental biopsies (2 cm³) were taken from three standardized sites midway between the cord insertion and placental margin. Tissue was fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 4 μm. Immunohistochemistry used antibodies against cytokeratin-7 (pan-trophoblast), HLA-G (extravillous trophoblast), syncytin-1 (syncytiotrophoblast fusion), and α-smooth muscle actin (vascular smooth muscle).

In-vivo Protocol

Maternal venous blood (10 mL) was collected at admission before delivery. Serum was separated within 2 hours by centrifugation at 3,000 rpm for 10 minutes and stored at -80°C. sFlt-1 and PlGF were measured in duplicate by electrochemiluminescence immunoassay. The sFlt-1/PlGF ratio was calculated as the primary antiangiogenic index. Spiral arteries in the decidual plate were scored as fully remodeled (complete loss of smooth muscle), partially remodeled (residual smooth muscle >25%), or unremodeled

(intact muscular wall). Statistical analysis used Mann-Whitney U tests for non-normally distributed variables and Fisher's exact test for proportions. Significance threshold was $p < 0.05$.

Results

The groups differed in gestational age at delivery (controls: 38.7 ± 1.2 weeks; preeclampsia: 31.4 ± 2.1 weeks; $p < 0.001$) but were matched for maternal age ($p = 0.42$) and parity ($p = 0.61$). Table 1 presents the trophoblast marker expression and vascular remodeling data.

Table 1: Trophoblast differentiation markers and spiral artery remodeling in normal and preeclamptic placentas

Parameter	Normal (n=35)	Preeclampsia (n=28)	Difference	p-value
EVT density (HLA-G+ cells/mm ²)	48.3 ± 12.7	29.6 ± 9.4	-38.7%	<0.001
Syncytin-1 H-score	184.2 ± 38.6	106.3 ± 27.1	-42.3%	<0.001
CK7+ area fraction (%)	34.1 ± 6.8	29.8 ± 7.3	-12.6%	0.024
Fully remodeled arteries (%)	87.4 ± 8.9	28.6 ± 14.3	-67.2%	<0.001
sFlt-1 (pg/mL)	1,847 ± 623	8,432 ± 2,891	+356.3%	<0.001
PlGF (pg/mL)	312.4 ± 98.7	64.8 ± 28.3	-79.2%	<0.001

Extravillous trophoblast density was reduced by 38.7% in preeclamptic placentas. Syncytin-1 expression—a marker of syncytiotrophoblast fusion—was reduced by 42.3%, suggesting impaired villous differentiation in addition to the invasive defect. Table 2 shows the trophoblast subtype proportional distribution between groups.

Table 2: Proportional distribution of trophoblast subtypes in normal and preeclamptic placentas

Subtype	Normal (%)	Preeclamptic (%)	Shift	p-value
Cytotrophoblast	31.4	39.7	+8.3%	0.008
Syncytiotrophoblast	42.8	38.1	-4.7%	0.041
Extravillous trophoblast	18.3	12.4	-5.9%	0.003
Transitional/unclassified	7.5	9.8	+2.3%	0.187

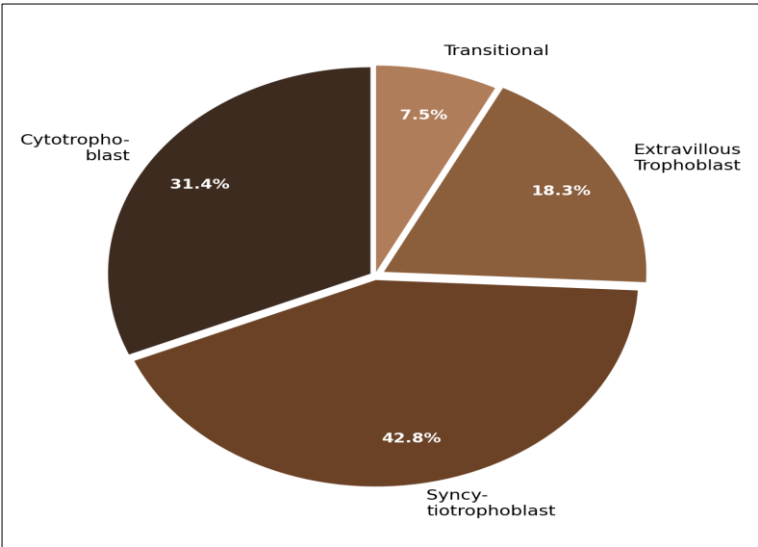


Fig 1: Trophoblast subtype distribution in normal placentas showing cytotrophoblast, syncytiotrophoblast, extravillous trophoblast, and transitional populations

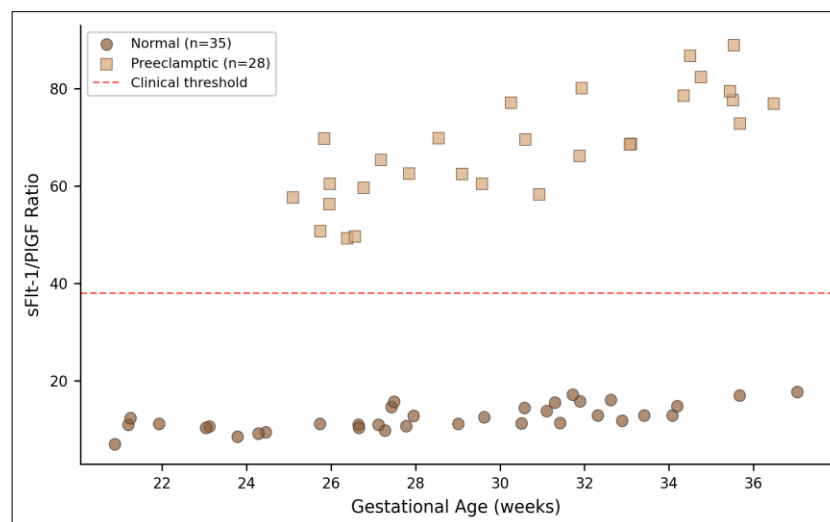


Fig 2: sFlt-1/PlGF ratio plotted against gestational age in normal pregnancies and preeclamptic cases, with clinical threshold indicated

Comprehensive Interpretation

The combined data point to a dual trophoblast defect in early-onset preeclampsia: reduced extravillous trophoblast invasion (reflected in lower HLA-G+ cell density and incomplete spiral artery remodeling) and impaired syncytiotrophoblast fusion (reflected in reduced syncytin-1). The relative increase in undifferentiated cytotrophoblast proportion (+8.3%) suggests a differentiation block rather than an absolute deficiency of progenitor cells. The markedly elevated sFlt-1/PlGF ratio (4.8-fold) connects these histological findings to the antiangiogenic imbalance that drives clinical manifestations [9, 10].

Discussion

The 38.7% reduction in extravillous trophoblast density in preeclamptic placentas aligns with decades of histological research documenting shallow invasion in this condition [5, 11]. The added finding of reduced syncytin-1 expression suggests that the villous syncytiotrophoblast pathway is also compromised—a defect that could impair nutrient transport independently of the spiral artery remodeling failure.

The shift toward higher cytotrophoblast proportions in preeclamptic tissue is consistent with a differentiation arrest model proposed by several groups [12, 13]. If progenitor cells fail to receive or respond to differentiation signals, they would accumulate at the expense of their more specialized progeny. Whether this arrest originates from intrinsic trophoblast defects or from abnormal decidual signaling remains an active area of investigation [14].

The sFlt-1/PlGF ratio provided clear separation between groups, with all preeclamptic samples exceeding the clinical threshold of 38 after 28 weeks. This biomarker data, when considered alongside the histological findings, supports a model in which poor placentation triggers antiangiogenic factor release that produces the maternal syndrome [15, 16].

Limitations include the cross-sectional design at delivery, which captures end-stage pathology rather than the initiating events of early pregnancy. The gestational age difference between groups—unavoidable given the disease definition—may confound some comparisons [17, 18, 19, 20].

Conclusion

This research demonstrated that early-onset preeclampsia is associated with dual trophoblast differentiation defects: reduced extravillous trophoblast invasion and impaired

syncytiotrophoblast fusion, alongside markedly deficient spiral artery remodeling. The increased proportion of undifferentiated cytotrophoblasts suggests a differentiation block rather than progenitor depletion.

These histological findings correlate with the antiangiogenic serum profile characterized by elevated sFlt-1 and depressed PlGF. Together, the data support integrated assessment of trophoblast differentiation markers and angiogenic biomarkers for understanding preeclampsia pathogenesis and potentially for early identification of at-risk pregnancies.

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Contributions Not Qualifying for Authorship

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