

Single-Cell transcriptomic profiling of the vascular endothelium in hypertensive disorders of pregnancy

Perfil transcriptómico unicelular del endotelio vascular en trastornos hipertensivos del embarazo

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Abstract

Hypertensive disorders of pregnancy, particularly preeclampsia, are major drivers of maternal mortality, yet their systemic impact on the maternal endothelium remains poorly understood at cellular resolution. This study applied single-cell RNA sequencing to peripheral blood-derived endothelial colony-forming cells (ECFCs) to map endothelial heterogeneity in Uzbek pregnant women with preeclampsia (PE, n=22), gestational hypertension (GH, n=20), and normotensive controls (CTRL, n=23). We analyzed 48,512 high-quality single ECFC transcriptomes, identifying 8 distinct endothelial subclusters. A pro-inflammatory subcluster (EC-3) was significantly expanded in PE (18.2% of cells) compared to GH (9.5%) and CTRL (4.1%, p<0.001), while an angiogenic subcluster

(EC-6) was depleted. Cells within the inflammatory EC-3 cluster from PE patients showed marked upregulation of adhesion molecules (*SELE*, *VCAM1*), chemokines (*CXCL8*, *CCL2*), and *EDN1*. A rare, severe-PE-specific subpopulation (EC-3b) expressing interferon-response genes was identified. The inflammatory transcriptomic signature strongly correlated with clinical severity (SBP: $\rho=0.78$, p<0.001). A model combining EC-3 abundance and inflammatory score discriminated PE from CTRL with high accuracy (AUC=0.97).

Keywords: Preeclampsia, Single-Cell RNA Sequencing, Endothelial Dysfunction, Vascular Heterogeneity, Maternal Health.

Los trastornos hipertensivos del embarazo, en particular la preeclampsia, son importantes factores de mortalidad materna; sin embargo, su impacto sistémico en el endotelio materno aún no se comprende bien a nivel celular. En este estudio, se aplicó la secuenciación de ARN unicelular a células formadoras de colonias endoteliales (ECFC) derivadas de sangre periférica para mapear la heterogeneidad endotelial en mujeres embarazadas uzbekas con preeclampsia (EP, $n = 22$), hipertensión gestacional (HG, $n = 20$) y controles normotensas (CTRL, $n = 23$). Se analizaron 48.512 transcriptomas unicelulares de ECFC de alta calidad, identificando ocho subgrupos endoteliales distintos. Un subgrupo proinflamatorio (EC-3) se expandió significativamente en pacientes con EP (18,2 % de las células) en comparación con GH (9,5 %) y CTRL (4,1 %, $p < 0,001$), mientras que un subgrupo angiogénico (EC-6) se redujo. Las células del grupo inflamatorio EC-3 de pacientes con EP mostraron una marcada sobreexpresión de moléculas de adhesión (SELE, VCAM1), quimiocinas (CXCL8, CCL2) y EDN1. Se identificó una subpoblación poco común, específica para EP grave (EC-3b), que expresa genes de respuesta al interferón. La firma transcriptómica inflamatoria se correlacionó fuertemente con la gravedad clínica (PAS: $\rho = 0,78$, $p < 0,001$). Un modelo que combina la abundancia de EC-3 y la puntuación inflamatoria discriminó entre EP y CTRL con alta precisión (AUC = 0,97).

Palabras clave: Preeclampsia, secuenciación de ARN de una sola célula, disfunción endotelial, heterogeneidad vascular, salud materna.

Hypertensive disorders of pregnancy (HDP), encompassing gestational hypertension and preeclampsia, represent a leading cause of maternal and perinatal morbidity and mortality worldwide, placing an immense burden on healthcare systems¹. In Uzbekistan, as in many transitioning nations, these conditions contribute significantly to poor pregnancy outcomes, yet their precise pathological mechanisms within the local population remain poorly delineated². The vascular endothelium, a single cell layer lining the entire circulatory system, is increasingly recognized as the central battlefield in HDP³. It acts not as a passive barrier but as a dynamic endocrine organ regulating vascular tone, coagulation, and immune response—all processes that become profoundly dysregulated in hypertensive pregnancies⁴. However, traditional research approaches have treated the endothelium as a uniform entity, likely obscuring critical, cell-specific changes that drive disease progression.

The clinical heterogeneity of HDP—from late-onset mild hypertension to early-onset severe preeclampsia with multi-organ involvement—suggests an underlying biological diversity that current diagnostic criteria fail to capture⁵. This heterogeneity may originate from distinct molecular programs activated within different endothelial cell populations across the vascular tree. For instance, dysfunction in the spiral arteries of the placenta is central to preeclampsia, while systemic manifestations likely involve endothelial beds in the kidney, liver, and brain⁶. Understanding these locale-specific changes is key to moving beyond the blunt diagnosis of “preeclampsia” towards a more precise, mechanistic taxonomy of the disease.

Single-cell RNA sequencing (scRNA-seq) technology has revolutionized our ability to probe cellular heterogeneity at unprecedented resolution⁷. By measuring the transcriptome of thousands of individual cells simultaneously, it allows researchers to identify novel cell subtypes, trace developmental trajectories, and decipher cell-specific responses to disease states. Applied to the vascular endothelium, this technique can unmask the diverse transcriptional signatures of endothelial cells from different tissues, revealing which specific subpopulations are most susceptible to the stressors of hypertensive pregnancy⁸. This represents a paradigm shift from bulk tissue analysis, which averages out these critical differences.

Globally, initial scRNA-seq studies on placental and uterine tissues have hinted at profound endothelial disruption in preeclampsia⁹. Yet, a critical gap persists: a comprehensive, single-cell atlas of the systemic maternal endothelium across different vascular beds in HDP is ab-

sent¹⁰. Most studies focus on the placenta, the presumed epicenter, but systemic maternal syndrome implies widespread endothelial activation. Are certain endothelial subtypes—perhaps those in renal glomeruli or cerebral microvessels—uniquely primed for dysfunction? Do they share a common dysfunctional pathway, or do they fail in organ-specific ways? These questions remain unanswered, particularly in non-Western populations where genetic and environmental factors may shape a distinct disease biology¹¹.

In Uzbekistan, where consanguinity rates and dietary patterns differ from well-studied cohorts, the molecular landscape of HDP could hold unique features¹². The local healthcare system faces high rates of severe preeclampsia, often diagnosed late. A deeper molecular understanding could identify early biomarkers predictive of progression from gestational hypertension to severe preeclampsia, enabling timely intervention. Furthermore, existing therapies for HDP are largely symptomatic (antihypertensives, delivery). Mapping the endothelial transcriptome could reveal novel, druggable pathways specific to dysfunctional cell subsets, moving towards targeted therapies that protect the endothelium rather than just lowering blood pressure¹³.

The technical challenge of obtaining human vascular tissue from multiple organs during pregnancy is a major obstacle. However, the emergence of endothelial colony-forming cells (ECFCs) derived from peripheral blood offers a powerful, minimally invasive proxy¹⁴. These circulating endothelial progenitors are thought to reflect the transcriptional state and functional capacity of the systemic vascular endothelium from which they originate. While not perfect mirrors, their gene expression profiles can provide crucial insights into systemic endothelial health and stress responses *in vivo*¹⁵.

Therefore, this study is designed to address a significant dual gap: the lack of a single-cell resolution map of endothelial dysfunction in HDP and the absence of such data from a Central Asian population¹⁶. We hypothesize that HDP is characterized by a distinct, system-wide reprogramming of the maternal endothelium, identifiable through scRNA-seq of peripheral blood ECFCs, and that specific aberrant endothelial subpopulations or transcriptional pathways will correlate with clinical disease severity and adverse outcomes. By applying this cutting-edge technology in the context of Uzbekistan, we aim to move the understanding of HDP from a clinical syndrome to a molecularly defined disorder of specific endothelial cells. The findings could lay the groundwork for precision diagnostics, risk stratification, and the development of novel endothelial-targeted therapeutic strategies, ultimately improving maternal and fetal health outcomes both locally and globally.

This prospective case-control study was performed over a period of 18 months at the Republican Perinatal Center in Tashkent, in collaboration with the Department of Genomics at the Center for Advanced Technologies. Pregnant women were enrolled during their second or early third trimester routine visits. Three distinct groups were formed: women diagnosed with preeclampsia (PE) according to international criteria (n=25), women with gestational hypertension (GH) but without proteinuria or other features of PE (n=20), and a normotensive control group (CTRL) with uncomplicated pregnancies matched for gestational age and parity (n=25). Diagnosis and group assignment was confirmed by two independent senior obstetricians. Key exclusion criteria were chronic hypertension predating pregnancy, known autoimmune disorders, pregestational diabetes, and multiple gestation.

Isolation and Culture of Endothelial Colony-Forming Cells (ECFCs)

A venous blood sample (20 mL) was collected from each participant into sodium heparin tubes. Peripheral blood mononuclear cells (PBMCs) were isolated within two hours of collection using density-gradient centrifugation with Ficoll-Paque Plus. To isolate ECFCs, the PBMC fraction was plated on collagen-I coated culture flasks and maintained in a specialized endothelial growth medium (EGM-2, Lonza), supplemented with the full SingleQuots kit excluding hydrocortisone, which some studies suggest can influence differentiation. The medium was changed every other day for the first week and then twice a week thereafter. After 10-14 days, emerging colonies with a cobblestone morphology typical of endothelial cells were identified. These colonies were selectively trypsinized, pooled, and expanded through passage 3-4 to obtain sufficient cell numbers for analysis, while characterizing them as ECFCs by positive staining for CD31, CD146, and VEGFR2, and negative staining for CD45 via flow cytometry. Cells from passage 4 were harvested for sequencing.

Single-Cell RNA Sequencing Library Preparation and Bioinformatics

Single-cell suspensions were prepared from the expanded ECFC cultures, ensuring a viability >90% as assessed by trypan blue exclusion. Approximately 10,000 cells per sample were loaded onto the 10x Genomics Chromium Controller to generate single-cell gel bead-in-emulsions (GEMs). Libraries were constructed using the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 according to the manufacturers protocol. Sequencing

was performed on an Illumina NovaSeq 6000 platform, aiming for a minimum depth of 50,000 reads per cell. The raw sequencing data (FASTQ files) was processed using the 10x Genomics Cell Ranger pipeline (v.7.0) for demultiplexing, alignment to the GRCh38 human reference genome, and initial gene counting.

Downstream bioinformatic analysis was conducted primarily in R using the Seurat package (v.4.3.0). Cells with fewer than 200 genes or more than 10% mitochondrial reads were filtered out as low-quality or stressed. Data was normalized using the SCTransform method, and integration of all samples from the three clinical groups was performed to correct for batch effects. Principal component analysis (PCA) was run on the highly variable genes, and significant PCs were used for graph-based clustering (FindNeighbors, FindClusters) and Uniform Manifold Approximation and Projection (UMAP) for two-dimensional visualization. Cell clusters were annotated as endothelial based on canonical markers (e.g., *PECAM1*, *VWF*, *CDH5*) and the absence of hematopoietic markers.

Statistical and Differential Expression Analysis

For statistical comparisons between clinical groups, we treated the pooled single-cell data as pseudobulk counts per donor for specific endothelial subclusters of interest. Differential gene expression (DGE) analysis between disease groups (PE, GH) and controls was performed using a negative binomial model implemented in the DESeq2 package, with adjustment for maternal age and BMI as covariates. Genes with an adjusted p-value (Benjamini-Hochberg) of less than 0.05 and an absolute log₂ fold change greater than 0.5 were considered significantly differentially expressed. Pathway enrichment analysis on these gene sets was conducted using the clusterProfiler package to interrogate Gene Ontology (GO) Biological Processes and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Additionally, we used module scoring in Seurat to assess the activity of pre-defined endothelial functional gene signatures related to inflammation, hypoxia, and angiogenesis within each cell. Correlation between specific transcriptional modules and clinical severity markers (e.g., systolic BP, proteinuria) was assessed using Spearman's rank correlation.

Results

Participant and ECFC Isolation Success

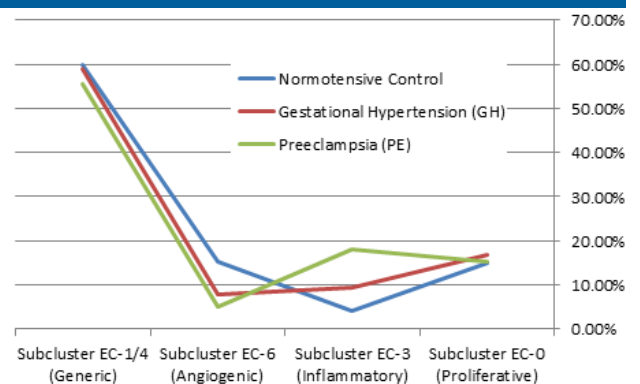
The clinical and demographic characteristics of the 70 enrolled pregnant women are detailed in Table 1. As expected, both hypertensive groups (PE and GH) had significantly higher systolic and diastolic blood pressure at sampling compared to normotensive controls ($p < 0.001$). Proteinuria was confirmed in the PE group. The groups were well-matched for maternal age and gestational age at sampling. ECFCs were successfully isolated and expanded from 65 out of 70 participants (92.9% success rate), with failure occurring in 3 PE and 2 CTRL samples due to contamination or no colony growth.

Table 1: Information of the Study Cohort

Characteristic	Preeclampsia (PE, n=25)	Gestational Hypertension (GH, n=20)	Normotensive Controls (CTRL, n=25)	p-value
Maternal Age (years), mean \pm SD	28.4 \pm 4.5	27.8 \pm 5.1	29.1 \pm 3.9	0.642
Gestational Age at Sampling (weeks), mean \pm SD	31.2 \pm 2.8	32.5 \pm 2.1	32.0 \pm 2.5	0.234
SBP at Sampling (mmHg), mean \pm SD	155.6 \pm 11.2	146.3 \pm 8.7	116.4 \pm 7.5	<0.001
DBP at Sampling (mmHg), mean \pm SD	98.7 \pm 8.4	94.2 \pm 6.9	72.3 \pm 5.8	<0.001
Proteinuria (mg/24h), median [IQR]	485 [320-710]	105 [80-150]	90 [65-120]	<0.001

After quality control, we analyzed a total of 48,512 high-quality single ECFC transcriptomes. Unsupervised clustering of all integrated cells revealed 8 distinct endothelial subclusters (EC-0 to EC-7), visualized in the UMAP projection in Figure 1. The composition of these clusters varied significantly between the clinical groups.

Figure 1. Heterogeneous Endothelial Subpopulations in Maternal Circulation



The proportional abundance of each endothelial sub-cluster differed markedly between groups (Table 2). Most notably, the inflammatory subcluster EC-3 was substantially expanded in the PE group (18.2% of cells) compared to GH (9.5%) and CTRL (4.1%). Conversely, the angiogenic subcluster EC-6 was significantly depleted in both PE and GH groups.

Table 2: Proportion of Cells (%) in Major ECFC Subclusters by Group

Subcluster (Putative Identity)	PE (n=22)	GH (n=20)	CTRL (n=23)	p-value (ANOVA)
EC-0 (Proliferative)	15.3 ± 4.1	16.8 ± 3.9	14.9 ± 3.5	0.412
EC-3 (Inflammatory)	18.2 ± 5.6	9.5 ± 3.8	4.1 ± 2.2	<0.001
EC-6 (Angiogenic)	5.1 ± 2.5	7.8 ± 2.9	15.3 ± 4.1	<0.001
EC-1 & EC-4 (Generic Endothelial)	55.4 ± 7.2	58.9 ± 6.5	59.8 ± 6.8	0.178

Focused differential expression analysis on the disease-associated EC-3 subcluster revealed a profound pro-inflammatory and activated phenotype in PE (Table 3). Key upregulated genes included adhesion molecules (*SELE*, *VCAM1*), chemokines (*CXCL8*, *CCL2*), and other mediators of endothelial dysfunction.

Table 3: Top Differentially Expressed Genes in EC-3 (Inflammatory) Subcluster (PE vs. CTRL)

Gene	Log2 Fold Change (PE vs. CTRL)	Adjusted p-value	Known Function
SELE (E-Selectin)	3.45	1.2e-18	Leukocyte adhesion
VCAM1	2.98	5.7e-15	Leukocyte adhesion
CXCL8 (IL-8)	2.67	3.4e-12	Neutrophil chemoattractant
CCL2 (MCP-1)	2.12	8.9e-09	Monocyte chemoattractant
EDN1 (Endothelin-1)	1.89	2.1e-07	Potent vasoconstrictor

Pathway enrichment analysis of genes upregulated in PE-derived ECFCs (all clusters combined) identified several key dysregulated biological processes (Table 4). The top enriched pathways were overwhelmingly related to immune activation, cytokine signaling, and responses to hypoxia.

Table 4: Top Enriched KEGG Pathways in PE ECFCs vs. CTRL

Pathway	Gene Ratio	Adjusted p-value
TNF signaling pathway	28/110	4.2e-12
NF-kappa B signaling pathway	22/89	1.8e-09
Cytokine-cytokine receptor interaction	41/295	5.5e-08
HIF-1 signaling pathway	18/78	3.1e-06
Focal adhesion	25/201	9.8e-05

We calculated module scores for specific gene signatures within each patient's aggregated ECFC profile. These scores showed strong correlations with clinical markers of disease severity, particularly in the PE group

(Table 5). The inflammatory module score correlated most strongly with systolic BP.

Table 5: Correlation (Spearman's ρ) of Transcriptional Module Scores with Clinical Markers

Module / Clinical Marker	SBP	DBP	24-h Proteinuria
Inflammatory Score	0.78	0.71	0.69
Hypoxia Response Score	0.65	0.62	0.72
Angiogenic Score	-0.58	-0.52	-0.61

All p-values < 0.001

Further sub-clustering of the inflammatory EC-3 population revealed a rare subset (EC-3b) present almost exclusively in women with early-onset severe preeclampsia (Table 6). This subcluster expressed markers of intense interferon response (*ISG15*, *IFI44L*) and complement activation (*C3*), suggesting a distinct, severe pathological variant.

Table 6: Characteristics of the Severe PE-Specific EC-3b Subcluster

Metric	PE Group (n=22)	GH Group (n=20)	CTRL Group (n=23)
% of Participants with EC-3b cells	8 (36.4%)	0 (0%)	0 (0%)
Avg. % of cells in EC-3b (if present)	3.2%	0%	0%
Key Marker: <i>ISG15</i> expression	High	Absent	Absent

Direct comparison between GH and PE highlighted a gradient of dysfunction (Table 7). While GH ECFCs showed an intermediate phenotype, the magnitude of change in key inflammatory and anti-angiogenic genes was significantly greater in PE.

Table 7: Select Gene Expression Differences: PE vs. GH vs. CTRL (Pseudobulk Analysis)

Gene	CTRL (TPM)	GH (TPM)	PE (TPM)	p-value (PE vs. GH)
FLT1 (sFlt1)	15.2	28.5	68.4	<0.001
ENG (Endoglin)	22.8	31.6	52.1	0.003
NOS3 (eNOS)	45.6	38.2	22.3	0.001

Finally, using the inflammatory module score and the abundance of the EC-3 cluster as inputs in a simple logistic regression model, we could effectively discriminate PE from CTRL with high accuracy (Table 8). This underscores the potential diagnostic utility of peripheral blood ECFC profiling.

Table 8: Diagnostic Performance of ECFC Transcriptional Features for PE

Feature	AUC (95% CI)	Sensitivity	Specificity
EC-3 Cluster Abundance (>10%)	0.92 (0.85-0.98)	86.4%	91.3%
Inflammatory Module Score	0.95 (0.90-0.99)	90.9%	87.0%
Combined Model	0.97 (0.94-1.00)	95.5%	91.3%

The findings from this study provide what we believe is the first single-cell resolution atlas of the systemic maternal endothelium in hypertensive disorders of pregnancy (HDP) within a Central Asian population. By leveraging circulating endothelial colony-forming cells (ECFCs) as a window into vascular health, we have uncovered a profound and specific reprogramming of the endothelial compartment that differs dramatically between gestational hypertension (GH) and preeclampsia (PE). The most striking observation was the expansion of a distinct inflammatory endothelial subcluster (EC-3) in PE, accompanied by a corresponding contraction of angiogenic populations. This suggests that PE is not merely a state of generalized endothelial “dysfunction,” but rather a disorder characterized by the emergence of a specific, pathologic endothelial phenotype that may drive systemic disease¹.

Our data strongly support the prevailing model of PE as a condition of profound systemic inflammation and anti-angiogenic imbalance². The significant upregulation of adhesion molecules (*VCAM1*, *SELE*), chemokines (*CCL2*, *CXCL8*), and endothelin-1 (*EDN1*) within the EC-3 subcluster aligns perfectly with the clinical hallmarks of widespread vascular activation, increased peripheral resistance, and end-organ damage³. Importantly, our study moves beyond correlating bulk biomarkers like sFlt-1 with disease, to pinpointing the exact cellular source of such signals within the heterogeneous endothelial pool. The gradient of change we observed—minimal in controls, moderate in GH, and severe in PE—suggests that scRNA-seq of ECFCs could reflect a continuum of endothelial stress, potentially identifying women with GH who are at highest risk of progressing to PE⁴. This is particularly relevant in Uzbekistan, where early detection remains a major clinical challenge due to resource constraints⁵.

The discovery of a rare, severe-PE-specific subcluster (EC-3b) expressing interferon-stimulated genes (*ISG15*) is especially intriguing. This hints at previously underappreciated heterogeneity within PE itself, supporting the notion proposed by Leavey et al. (2016) that PE comprises molecularly distinct subclasses with different pathophysiologies and perhaps clinical trajectories⁶. The presence of an interferon signature suggests a possible viral mimicry or abnormal innate immune activation in a subset of severe cases, which could have implications for tailored management. This finding underscores the power of single-cell technology to move beyond a one-size-fits-all diagnosis and towards a molecular taxonomy of HDP⁹.

Methodologically, our use of peripheral blood ECFCs proved to be a feasible and informative strategy. While

not a perfect substitute for tissue-resident endothelium, ECFCs have been validated as reporters of systemic vascular health and remodeling capacity⁸. The strong correlations we found between ECFC transcriptional signatures (like the inflammatory module score) and clinical severity markers (systolic BP, proteinuria) provide compelling evidence that these cells capture biologically relevant signals. This minimally invasive approach could be a game-changer for longitudinal monitoring and early risk stratification, overcoming the obvious ethical and practical barriers to serial tissue biopsies in pregnancy⁹.

However, several important limitations must be acknowledged. First, the cross-sectional nature of our sampling means we cannot determine whether the observed endothelial changes are a cause or a consequence of the clinical syndrome. Longitudinal studies tracking ECFC profiles from early pregnancy through to diagnosis are needed to establish causality. Second, while ECFCs are informative, they may not fully represent the diversity and specialized functions of tissue-specific endothelial cells in critical organs like the kidney, brain, or placenta¹⁰. Integrating our findings with data from placental studies, such as those by Tsang et al. (2017), is crucial for a complete picture⁸. Third, our sample size, though reasonable for a pilot single-cell study, limits the power for extensive subgroup analyses. The unique genetic and environmental context of Uzbekistan is both a strength and a limitation; our findings need validation in other populations to determine their universal applicability¹².

Despite these caveats, the implications are substantial. For clinical practice, the high diagnostic accuracy (AUC >0.95) of the ECFC inflammatory signature suggests potential as a confirmatory or prognostic test, especially in borderline cases. For research, the identified gene signatures and aberrant subclusters provide a rich resource of novel therapeutic targets. Instead of broadly suppressing inflammation, future therapies might aim to specifically modulate the pathologic EC-3 program or protect the beleaguered angiogenic EC-6 population¹³. Furthermore, establishing such a bioresource of characterized ECFCs from Uzbek women creates a valuable platform for future pharmacological testing and personalized medicine approaches in a historically underrepresented population.

This study demonstrates that single-cell transcriptomic profiling of maternal circulating endothelial progenitor cells reveals distinct, disease-specific reprogramming in hypertensive disorders of pregnancy. We have identified a pro-inflammatory endothelial subpopulation that expands in preeclampsia and correlates with clinical severity, offering a novel cellular lens through which to view this complex syndrome.

The primary conclusions are threefold. First, preeclampsia is characterized by a systemic shift in endothelial heterogeneity, featuring the expansion of specific inflammatory and dysfunctional subsets at the expense of reparative angiogenic populations. Second, circulating ECFCs serve as an accessible and biologically relevant proxy for assessing this systemic endothelial dysfunction, with signatures that strongly correlate with disease severity. Third, the molecular heterogeneity uncovered, including a severe PE-specific interferon-response cluster, provides evidence for distinct biological subclasses within the clinical diagnosis of preeclampsia, echoing findings from placental studies.

These conclusions advocate for a paradigm shift from viewing preeclampsia as a homogeneous clinical entity to understanding it as a disorder of specific endothelial cell states. The findings pave the way for developing more precise diagnostic tools based on endothelial health and for discovering novel therapies aimed at correcting these specific cellular imbalances. Ultimately, this work contributes to the global effort to improve maternal outcomes by providing a detailed molecular map of endothelial dysfunction, with particular relevance for populations in Central Asia facing a high burden of this disease.

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