

ORIGINAL ARTICLE



Senescent Syncytiotrophoblast Secretion During Early Onset Preeclampsia

Olivia Nonn¹*, Olivia Debnath*, Daniela S. Valdes¹*, Katja Sallinger, Ali Kerim Secener¹, Cornelius Fischer¹, Sebastian Tiesmeyer¹, Jose Nimo¹, Thomas Kuenzer¹, Juliane Ulrich, Theresa Maxian¹, Martin Knöfler, Philipp Karau¹, Hendrik Bartolomaeus¹, Thomas Kroneis¹, Alina Frolova¹, Lena Neuper, Nadine Haase, Alexander Malt, Niklas Müller-Bötticher¹, Kristin Kräker¹, Sarah Kedziora¹, Désirée Forstner¹, Roland Eils, Ruth Schmidt-Ullrich¹, Sandra Haider¹, Stefan Verlohren¹, Christina Stern¹, Meryam Sugulle¹, Stuart Jones¹, Basky Thilaganathan¹, Tu'uhevaha J. Kaitu'u-Lino¹, Stephen Tong, Berthold Huppertz¹, Amin El-Heliebi¹, Anne Cathrine Staff¹, Fabian Coscia¹, Dominik N. Müller, Ralf Dechend¹, Martin Gauster¹, Naveed Ishaque¹,† Florian Herse¹†

BACKGROUND: Preeclampsia is a severe hypertensive disorder in pregnancy that causes preterm delivery, maternal and fetal morbidity, mortality, and life-long sequelae. Understanding the pathogenesis of preeclampsia is a critical first step toward protecting mother and child from this syndrome and increased risk of cardiovascular disease later in life. However, effective early predictive tests and therapies for preeclampsia are scarce.

METHODS: To identify novel markers and signaling pathways for early onset preeclampsia, we profiled human maternal-fetal interface units (fetal villi and maternal decidua) from early onset preeclampsia and healthy controls using single-nucleus RNA sequencing combined with spatial transcriptomics. The placental syncytiotrophoblast is in direct contact with maternal blood and forms the barrier between fetal and maternal circulation.

RESULTS: We identified different transcriptomic states of the endocrine syncytiotrophoblast nuclei with patterns of dysregulation associated with a senescence-associated secretory phenotype and a spatial dysregulation of senescence in the placental trophoblast layer. Elevated senescence markers were validated in placental tissues of clinical multicenter cohorts. Importantly, several secreted senescence-associated secretory phenotype factors were elevated in maternal blood already in the first trimester. We verified the secreted senescence markers, PAI-1 (plasminogen activator inhibitor 1) and activin A, as identified in our single-nucleus RNA sequencing model as predictive markers before clinical preeclampsia diagnosis.

CONCLUSIONS: This indicates that increased syncytiotrophoblast senescence appears weeks before clinical manifestation of early onset preeclampsia, suggesting that the dysregulated preeclamptic placenta starts with higher cell maturation resulting in premature and increased senescence-associated secretory phenotype release. These senescence-associated secretory phenotype markers may serve as an additional early diagnostic tool for this syndrome. (**Hypertension. 2025;82:787–799. DOI: 10.1161/HYPERTENSIONAHA.124.23362.**) • **Supplement Material.**

Key Words: placenta ■ plasminogen activator inhibitor 1 ■ pre-eclampsia ■ pregnancy ■ pregnancy trimesters

See Editorial, pp 800-803

Correspondence to: Florian Herse, Max-Delbrück-Centrum für Molekulare Medizin in der Helmholtz-Gemeinschaft, Robert-Rössle-Straße 10, 13125 Berlin, Germany. Email florian.herse@charite.de

*O. Nonn, O. Debnath, and D.S. Valdes contributed equally.

†N. Ishaque and F. Herse co-supervised this work.

Supplemental Material is available at <https://www.ahajournals.org/doi/suppl/10.1161/HYPERTENSIONAHA.124.23362>.

For Sources of Funding and Disclosures, see page 798.

© 2024 The Authors. *Hypertension* is published on behalf of the American Heart Association, Inc., by Wolters Kluwer Health, Inc. This is an open access article under the terms of the [Creative Commons Attribution Non-Commercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use, distribution, and reproduction in any medium, provided that the original work is properly cited, the use is noncommercial, and no modifications or adaptations are made.

Hypertension is available at www.ahajournals.org/journal/hyp

NOVELTY AND RELEVANCE

What Is New?

We present a model that longitudinally reconstructs molecular events at the human maternal-fetal interface by single-nucleus RNA sequencing and spatial transcriptomics.

This model identifies a senescent phenotype early in pregnancy in early onset preeclampsia originating from the villous syncytiotrophoblast; the fetal barrier is in direct contact with maternal blood.

What Is Relevant?

These senescence-associated secretory phenotype factors are secreted into the maternal circulation and can interact with maternal vessels.

We provide evidence that maternal serum levels of senescence markers, activin A, PAI-1 (plasminogen activator inhibitor 1), and GDF15 (growth differentiation factor 15), contribute to a senescence-associated pathophysiology early in pregnancy, associated with early onset preeclampsia development later in gestation, thus representing markers for prediction and diagnosis.

What Are the Clinical/ Pathophysiological Implications?

Our study provides the basis for a novel understanding of the pathophysiology of early onset preeclampsia, which is centered around premature syncytiotrophoblast senescence. The causal link leading to this state should be further studied.

Symptomatic blood pressure treatment in preeclampsia and iatrogenic preterm delivery do not treat preeclampsia in its initial stages. Our model suggests that future preeclampsia therapy should focus on the senescence-associated pathophysiology early in pregnancy to prevent the development of a syndrome that poses a long-term individual and public health concern.

Nonstandard Abbreviations and Acronyms

DEG	differentially expressed gene
eoPE	early onset preeclampsia
EVT	extravillous trophoblast
FGR	fetal growth restriction
GDF15	growth differentiation factor 15
LEP	leptin
PAI-1	plasminogen activator inhibitor 1
SASP	senescence-associated secretory phenotype
scRNA-seq	single-cell RNA sequencing
snRNA-seq	single-nucleus RNA sequencing
UtA-PI	uterine artery pulsatility index
vSTB	villous endocrine syncytiotrophoblast

severe subvariant of preeclampsia and necessitates delivery before the 34th week of gestation. It also poses great risks to the child due to preterm birth.⁴ Moreover, there are long-term consequences of preeclampsia⁵ because it is associated with a reduced lifespan for both mother and child due to cardiovascular complications later in life.^{5,6}

Importantly, no effective therapies are known once preeclampsia has been established² and delivery is the only option to terminate the syndrome,² suggesting a central role of the placenta.⁷

Placental cells are of fetal origin and primarily consist of different types and differentiation stages of trophoblast cells. Factors shed from the preeclamptic placenta seem to cause maternal endothelial or vascular dysfunction, which then leads to hypertension and multiorgan injury in the mother (maternal syndrome).⁸ Most importantly, the syncytiotrophoblast that is in direct contact with maternal blood and forms the trophoblast barrier between the fetal and maternal circulation is suspected to play a crucial role in transferring placental dysregulation to the maternal syndrome.

Recent single-cell sequencing studies examining reproductive tissues from healthy women (from both pregnancy and nonpregnancy samples) have shed new insights into the maternal-fetal interface,^{9–12} trophoblast subtypes,^{13,14} and the endometrium¹⁵ before uncomplicated pregnancies. The maternal-fetal interface mediates in utero conditions, such as oxygen, nutrients, and

Hypertensive disorders in pregnancy, such as preeclampsia, account for a significant proportion of maternal deaths.¹ Current diagnosis relies only on clinical signs,² such as the presence of hypertension, accompanied by proteinuria or other maternal organ dysfunctions and often fetal growth restriction (FGR). Symptoms appear late in pregnancy, after 20 weeks of gestation, when the underlying pathology that will cause maternal and fetal morbidity is already advanced.³ Early onset preeclampsia (eoPE) is a

waste exchange, which facilitate a successful pregnancy and shape the life-long health of mother and child. While the single-cell landscape of healthy placentas has been well described, no comprehensive data exist from the uteroplacental tissue of patients with eoPE.

Our objective was to investigate the placental pathophysiology of eoPE by investigating both maternal decidua and fetal placental villi, with a particular focus on the syncytiotrophoblast. For novel insights, we validated our findings by analyzing maternal serum obtained before the clinical onset of the syndrome.

METHODS

Data Availability

Detailed protocols are provided in the [Supplemental Methods](#), including information on sample procurement, wet-laboratory and computational steps for single-nucleus RNA sequencing (snRNA-seq), and in situ sequencing preprocessing and downstream analyses. Details are also given for RNA isolation and quantitative real-time PCR (RT-qPCR) measurements, immunofluorescence and immunohistochemistry staining, enzyme linked immunosorbent assay (ELISA) measurements, and disease prediction models. Scripts for reproducing analyses and visualizations are available via https://github.com/HiDiHlabs/preeclampsia_Nonn_etal/. Anonymized data and materials

have been made publicly available, as described in detail in the [Supplemental Methods](#).

Additional data are provided in figures and tables in the [Supplemental Material](#).

Clinical and Multiomics Study Design

Our study design included comprehensive sampling of placental tissue taken across pregnancy (from different cases), data analyses, and multilevel validation (Figure 1). To reduce the heterogeneity of the study cohort ([Table S1](#)), we narrowed our inclusion criteria to eoPE with coexisting FGR. Furthermore, we restricted our preeclampsia cohort to those with proteinuric preeclampsia.

Given that FGR can occur without preeclampsia,² we aimed to exclude the possibility that FGR may result in confounding effects on the transcriptomic profile of preeclampsia. Indeed, gene expression data¹⁷ from eoPE with FGR (n=18) compared with eoPE without FGR (n=19) did not identify differentially expressed targets in the placental transcriptomes, suggesting that there is no confounding by the presence of FGR in our eoPE cohort ([Table S2](#)).

To study the temporal disease progression of preeclampsia, we obtained uteroplacental tissue samples for snRNA-seq from early (n=13, at 5–11 weeks of gestation; early control) and healthy full-term pregnancies (n=10, ≥38 weeks of gestation; healthy term pregnancy) and pregnancies complicated by eoPE (n=11, 27–33 weeks of gestation; Figure 1; [Table S1](#)). To adjust for the possible confounding of a preterm signature with the eoPE-specific snRNA-seq signature, we integrated

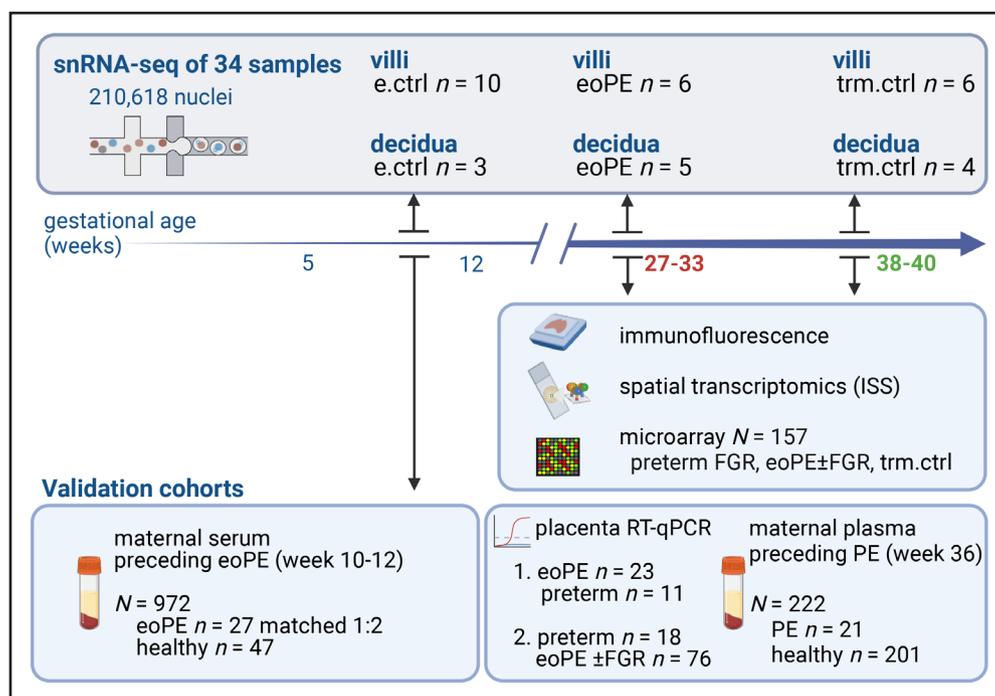


Figure 1. Multiomics study design using multiple validation levels with patient cohorts.

Schematic illustration of the experimental design across different stages of gestation, early pregnancy (e.ctrl), late healthy term pregnancy (trm.ctrl), and early onset preeclampsia (eoPE). Placental tissue was surgically sampled, and villi and decidua were collected separately for snRNA-seq. Tissues from e.ctrl correspond to 5 to 10 weeks of gestation, eoPE tissue was sampled at <34 weeks of gestation (range, 27–33 weeks of gestation), and trm.ctrl samples at >38 weeks (range, 38–40 weeks of gestation). The gestational age difference between healthy term controls (trm.ctrl) and diseased eoPE was corrected by using additional single-cell RNA sequencing (scRNA-seq)¹⁶ data from preterm (ptrm)-delivered nonhypertensive obstetric pathologies. All key findings were validated in patient cohorts using the methods outlined in the lower figures. FGR indicates fetal growth restriction; ISS, in situ sequencing; PE, preeclampsia; RT-qPCR, quantitative real-time PCR; and snRNA-seq, single-nucleus RNA sequencing.

a single-cell RNA sequencing (scRNA-seq) data set¹¹ that compared preterm with term births ($n=16$; [Table S1](#)). Potential confounding related to labor was eliminated by only including specimens from birth by cesarean section before labor. Furthermore, we used a bulk gene expression data set¹⁷ of placental villous tissue to compare signatures of placentas from nonpreeclamptic preterm and term deliveries. Thus, we were able to investigate the specific profile of eoPE in relation to non-hypertension-related preterm pathologies.

For validation, RT-qPCR was performed using placental specimens from cohorts obtained from 4 centers (preterm matched controls: $n=29$; eoPE: $n=84$; and eoPE+FGR: $n=17$; [Table S1](#)). Finally, secreted preeclampsia-related factors of interest were analyzed in maternal blood samples from 2 longitudinal cohorts in which blood was sampled before a preeclampsia diagnosis in early (controls: $n=47$; eoPE: $n=27$) and in late gestation (control: $n=201$; preeclampsia: $n=21$; [Table S1](#)).

Two-group baseline characteristic and experimental comparisons (healthy term pregnancy versus eoPE; preterm versus eoPE; and FGR versus noFGR) were statistically analyzed and visualized using the GraphPad Prism software (v9.4.1). Values were evaluated for normality using the Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests. Homoscedasticity plots were visually inspected per group to assess variance. Significance was tested between groups using either a 2-tailed unpaired *t* test with Welch correction or a 2-tailed Mann-Whitney *U* test, which does not assume equal variances.

RESULTS

snRNA-Seq Analysis

We profiled 34 placental samples at various stages of pregnancy (Figure 1). These samples were taken from the chorionic villi (villi as tissue of origin), which are the fetal part of the placenta, and the decidua basalis, the maternal part of the placenta.

Using the 10× Genomics Chromium platform, we sequenced and harmonized 95 253 nuclei of early control, 50 515 of term control (healthy term pregnancy), and 64 850 of eoPE samples (total, 210 618 nuclei), after low-quality nuclei were removed by extensive preprocessing measures, such as batch effect assessment and doublet nuclei inference (Figure 2A and 2C; [Figures S1 and S2](#), [Tables S3 through S7](#)).

Cell Types in Placenta and Decidua

We identified 24 decidual and 15 villous cell types and cell states of the immune, vascular endothelial, matrisome, and trophoblast compartments (Figure 2A and 2E; [Figures S4 and S5](#); [Table S8](#)). Variations in cell composition within the immune, vascular endothelial, matrisome, and trophoblast compartments in placental samples collected at the different gestation time points were evident (Figure 2D and 2E; [Figure S4](#); [Table S3](#)) and confirmed placental plasticity and functional adaptations at different stages of pregnancy.

As expected, the most abundant cell population in the villi were the various trophoblast types that exist in different cell/nucleus types and states, all of which originate from the proliferating mononucleated cytotrophoblast (Figure 2D and 2E; [Figure S4](#)). The cytotrophoblast is a bipotent progenitor, giving rise to the 2 major trophoblast differentiation lineages: the syncytiotrophoblast and the extravillous trophoblast (EVT) lineage (located in the decidua). EVT emerges from cell column trophoblasts that derive directly from cytotrophoblast. We identified 3 cell column trophoblast states, including proximal, distal, and transition state ([Figure S8](#)). The syncytiotrophoblast is generated by cytotrophoblast fusion and forms an endocrine syncytium, a multinuclear layer without lateral cell borders. Of note, we identified 3 different nuclei states, villous syncytiotrophoblast immature (vSTBim), villous endocrine syncytiotrophoblast (vSTB) 1, and vSTB2, within the villous (villi as tissue of origin) syncytiotrophoblast¹⁴ and found that the corresponding gene expression profiles represent progressing differentiation states along a pseudotime axis, namely, from vSTBim via vSTB1 and, finally, vSTB2 ([Figure S6](#); [Table S8](#)).

Of note, we found a significant decrease in the overall proportion of nuclei derived from the fetal villous immune compartment when comparing term controls with eoPE samples (Figure 2F).

Prerequisites and Corrections for snRNA-Seq Analysis

Using our snRNA-seq data, we proceeded to compare the specific gene expression profile of eoPE to term control placenta samples of healthy women ([Table S9](#)). All samples were derived from uteroplacental tissue either at 27 to 33 weeks of gestation for the eoPE cohort or at 38 to 40 weeks for healthy term controls (Figure 1; [Table S1](#)). Note that it is evidently not possible to collect placentas from healthy controls at the same gestational time point as the eoPE cohort because eoPE pregnancies are terminated prematurely, and there is no reason for premature delivery in the case of healthy pregnancies.¹⁸ Thus, to adjust for the possible confounding of a preterm gene signature that would interfere with the eoPE-specific snRNA-seq signature (Figure 1), we integrated a published scRNA-seq data set¹⁶ that compared preterm births for medical indications other than preeclampsia (ie, all remained normotensive throughout their pregnancies) with term births ($n=16$; [Figure S3](#); [Table S1](#)).

Transcriptomic Dysregulation of the Decidua in eoPE

When comparing eoPE with late-term controls, we identified 1791 and 1670 differentially expressed genes

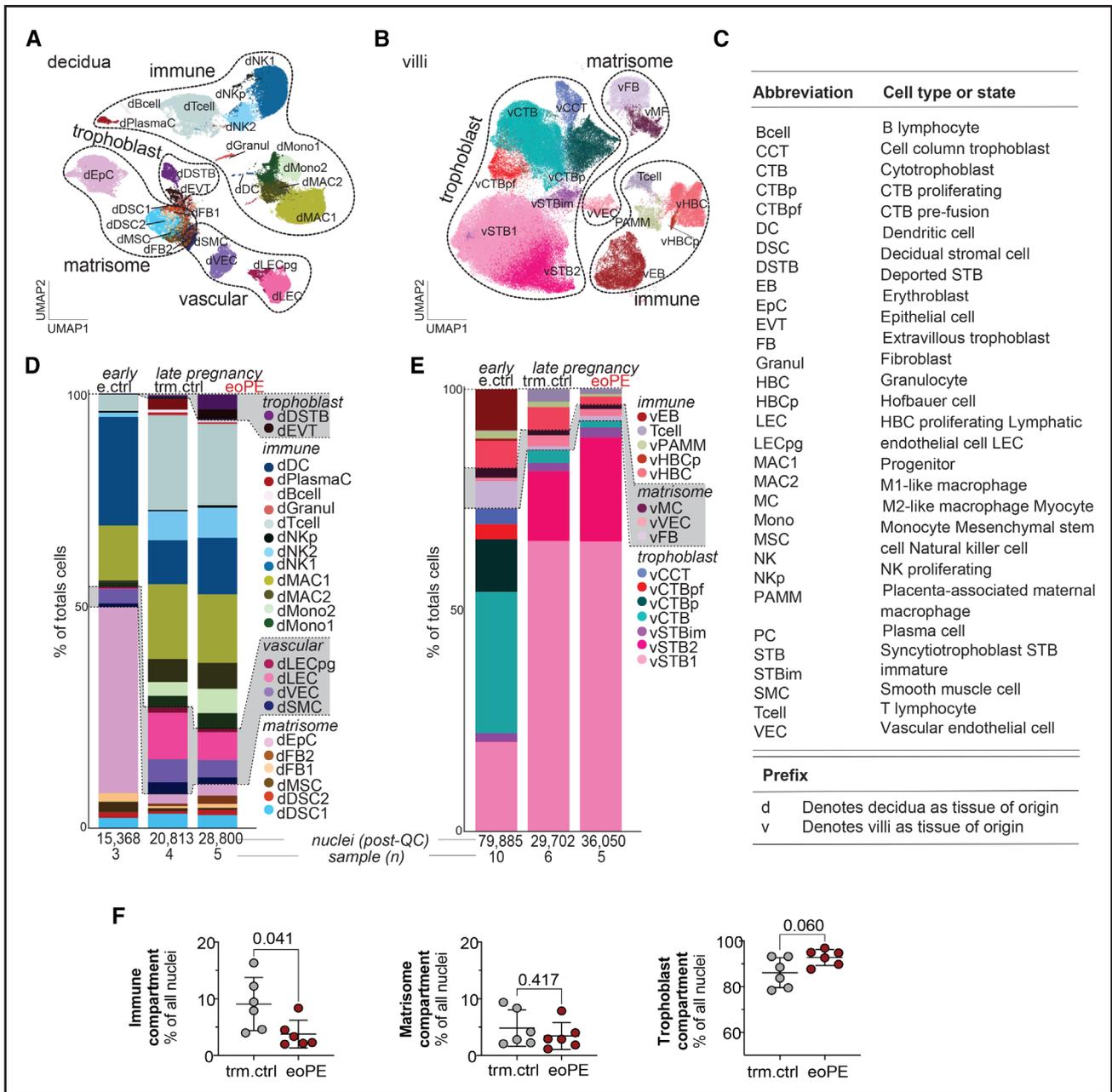


Figure 2. Single-nucleus-resolved transcriptomic landscape of the healthy and early onset preeclamptic maternal-fetal interface.

A and **B**, Uniform manifold approximation and projection (UMAP) visualizing (A) maternal (decidual, d) and (B) fetal (villous, v) cell types and states obtained from the single-nucleus RNA sequencing (snRNA-seq) data, with integrated samples from early pregnancy (e.ctrl), late healthy pregnancy (trm.ctrl), and early onset preeclampsia (eoPE). Each dot represents a nucleus; color indicates cell type or state. **C**, Abbreviations for cell types and states presented in this study. **D** and **E**, Distribution of cell type composition at different gestational time points for (D) decidua and (E) villi (% of total tissue nuclei). Numbers under the bars indicate the total high-quality nuclei and their corresponding biological replicate sample size. **F**, Cell composition comparison by trophoblast, matrisome, and immune compartments between term.ctrl. and eoPE. Two-tailed Welch *t* test.

(DEGs) in decidual and villous samples, respectively (Table S10).

Comparing term control with eoPE, the differential gene expression analysis of the decidua identified 899 genes in the immune compartment. These comprised genes identifying lymphoid lineages, such as natural killer (decidual natural killer 1 and decidual natural killer 2), decidual T cells, and myeloid lineages, including

macrophages (decidual M1-like macrophage and decidual M2-like macrophage) and monocytes (dMono1), as well as 472 genes of the matrisome compartment, including fibroblasts (dFB1/2 [decidual fibroblast cluster 1 and cluster 2]), decidual stromal cell 1, and decidual smooth muscle cells, and 679 genes of the endothelial compartment, including decidual vascular endothelial cells, decidual lymphatic endothelial cells, and decidual

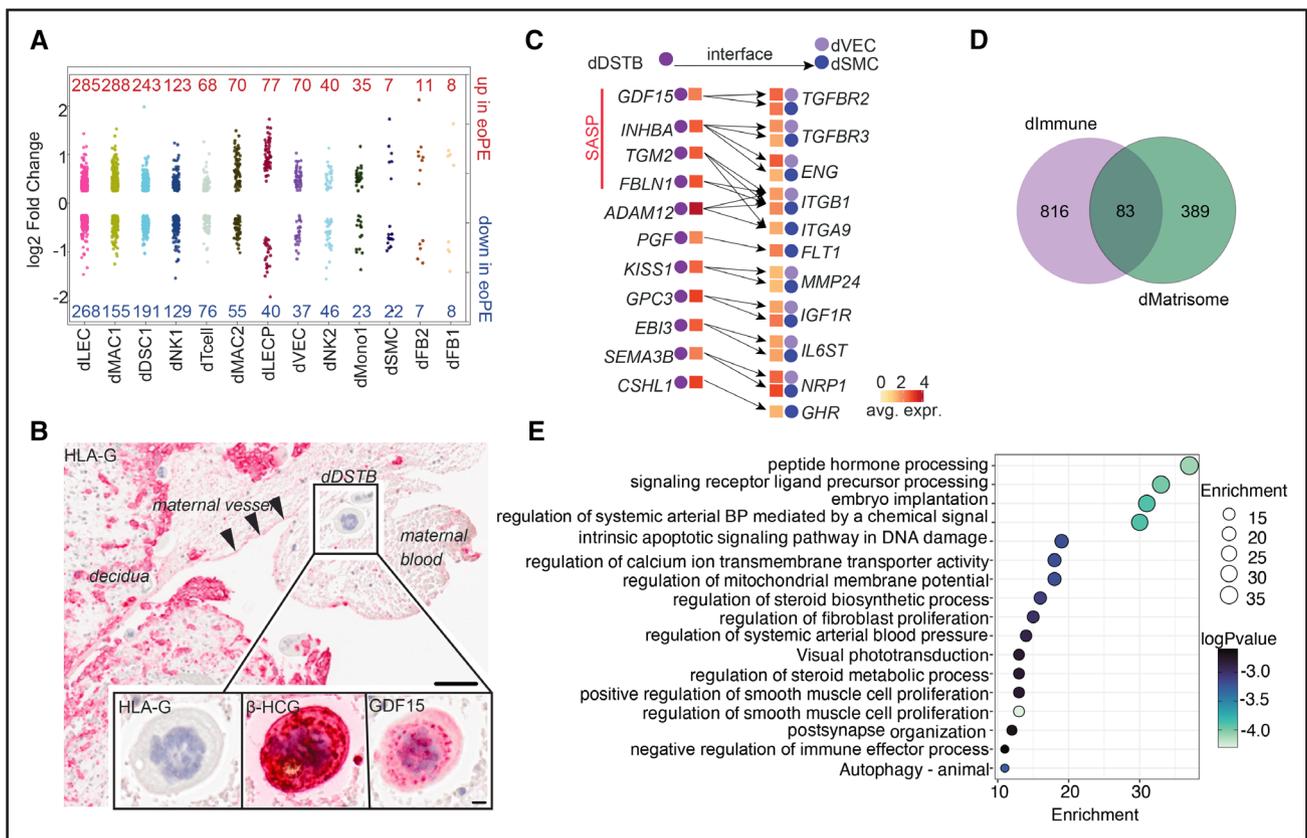


Figure 3. Early onset preeclampsia dysregulates the decidua and its interaction with fetal cells.

A, Significantly dysregulated gene expression profiles between term control (trm.ctrl) and early onset preeclampsia (eoPE) decidual cell types (\log_2 -fold change [\log_2 FC] $\geq \pm 0.25$; adjusted $P < 0.05$). \log_2 FC between conditions for each dysregulated gene (dot) visualized. The decidual epithelial cells, monocytes, proliferating natural killer cells, plasma cells, granulocytes, progenitor lymphatic endothelial cells, extravillous trophoblast, and departed syncytiotrophoblast were excluded due to large composition changes between trm.ctrl and eoPE. **B**, GDF15- protein in decidual departed syncytiotrophoblast (dDSTB) localized by immunohistochemistry in decidual tissue. dDSTB is identified among maternal erythrocytes in maternal vessels as an HLA-G negative (human leukocyte antigen G) β -hCG positive (human chorionic gonadotropin) GDF15 positive (growth differentiation factor 15) fragment (shown in serial sections' staining as indicated). Arrows indicate the maternal vessel border ($n=3$, representative area shown). Scale bars, 100 μ m (overview) and 20 μ m (detailed). **C**, Interaction between pathologically altered dDSTB-secreted ligands with decidual vascular endothelial cell (dVEC) and smooth muscle cell (dSMC) receptors at the maternal-fetal interface. Only altered receptor-ligand interaction pairs during eoPE are shown; arrows point toward receptors (Wilcoxon rank-sum test, $P < 0.05$, identified by multiple tools and databases). Dot colors encode cell types/states, and squares illustrate the average expression of receptors and ligands of interest. Senescence-associated secretory phenotypes (SASPs) are marked. **D**, Overlap of differentially expressed genes between decidual immune and matrisome cell-type groups. **E**, Pathway enrichment analysis of commonly dysregulated genes in decidual immune and matrisome compartments ($n=83$) calculated using the Metascape tool. In **A**, **C**, and **D**, $n=11$ villi (6 late controls and 5 eoPEs) and $n=9$ decidua (4 late controls and 5 eoPEs).

proliferating lymphatic endothelial cell (Figure 3A; Figure S7; Tables S3 and S9).

We also observed nuclei of fetal origin with syncytiotrophoblast profiles in the decidual samples (Figure 2A and 2D). As part of the epithelial turnover in the placenta, syncytiotrophoblast nuclei eventually undergo cellular aging and are shed and deported into the maternal blood.^{19,20} This suggests that transcriptomic syncytiotrophoblast profiles identified in decidua samples may correspond to syncytiotrophoblast nuclei shed from the placental villi into the maternal circulation and transported toward the decidua departed syncytiotrophoblast. We confirmed this finding by immunohistochemical analysis on decidual sections, which revealed multinucleated syncytial fragments expressing villous syncytiotrophoblast markers, GDF15 (growth differentiation factor 15) and β -hCG (human chorionic

gonadotropin), within the maternal vascular compartment, in the absence of EVT marker, HLA-G (human leukocyte antigen G; Figure 3B). Of note, syncytiotrophoblast-secreted ligands have high-affinity receptors in vascular endothelial and smooth muscle cells of the decidua; 36% of these ligands are associated with the senescence-associated secretory phenotype (SASP; Figure 3C). We also investigated the classical decidual-invading EVT, which interacts via *HLA-G*, *FN1*, and *SERPINE2* with receptors of various maternal decidual cell types (Figure S8).

Transcriptomic Dysregulation of the Placenta in eoPE

We next investigated the impact of eoPE in villi compared with term controls (Figure 4A; Table S5). The

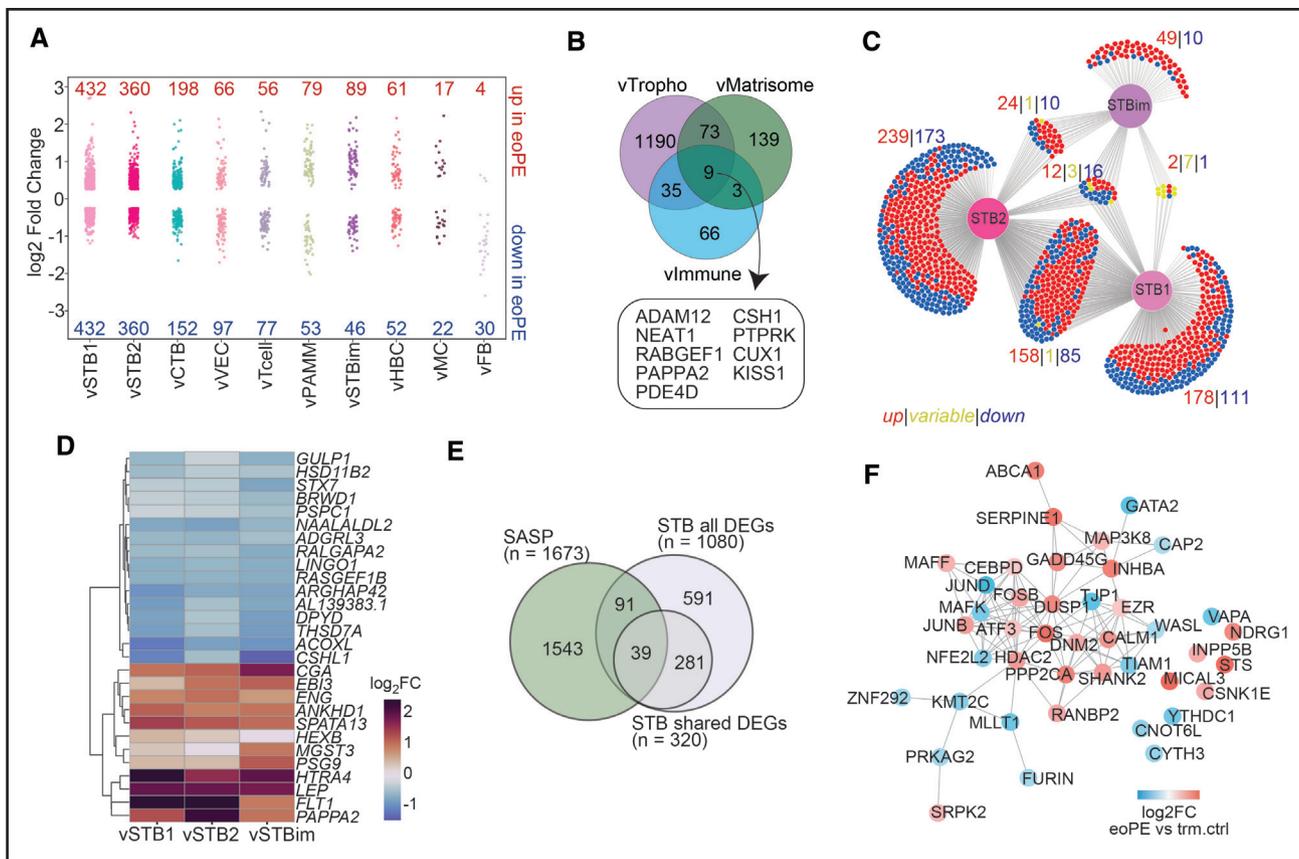


Figure 4. Syncytiotrophoblast populations are the most affected placental cell population in early onset preeclampsia.

A, Significantly dysregulated gene expression profiles between term control (trm.ctrl) and early onset preeclampsia (eoPE) villous cell types (\log_2 -fold change [\log_2FC] $\geq \pm 0.25$; adjusted $P < 0.05$). \log_2FC between conditions for each dysregulated gene (dot) visualized. **B**, Overlap of differentially expressed genes (DEGs) between villous cell-type groups. **C**, Convergence of DEGs in the nucleus states from the syncytiotrophoblast (STB) where each dot represents a single gene (shared upregulated: red; shared downregulated: blue; and differential gene expression: yellow). **D**, Heatmap of the mean \log_2FC values of overlapping genes of concordant dysregulation directionality ($n = 28$) between nuclear STB (vSTB) states as visualized in **C**. **E**, Overlap of senescence-associated secretory phenotype (SASP) genes derived from the human SASP atlas (Buch Institute) with all and shared genes dysregulated in vSTB nuclear states (min. \log_2FC 0.4, genes expressed in $\geq 30\%$ of nuclei and adjusted $P < 0.05$). **F**, Network of statistically significant hub genes of vSTB DEGs. Node color represents \log_2FC from the DEG between trm.ctrl and eoPE. Overall, $n = 12$ placentas (6 term controls and 6 eoPEs).

dysregulated profile of eoPE showed that gene expression in trophoblast cell types was the most dysregulated.

In line with this, eoPE caused a global dysregulation of gene expression, which is confirmed by overlapping profiles of trophoblasts with immune and meso-endothelial groups (Figure 4B). Nine genes were deregulated in all villous cell types and states compared with controls: *ADAM12*, *NEAT1*, *RABGEF1*, *PAPPA2*, *PDE4D*, *CSH1*, *PTPRK*, *CUX1*, and *KISS1*. Cell-type-specific dysregulation was observed in the villous trophoblast cell category (vSTB1, vSTB2, vSTBim, and villous cytotrophoblast [vCTB]) with 1307 genes, the matrisome compartment (villous vascular endothelial cell [vVEC], villous fibroblast [vFB], and vMC) with 224 genes, and in the immune cell compartment with 113 genes (villous Hofbauer cell [vHBC] and villous Tcells; Table S10). The top 5 DEGs per cell type are shown in Figure S7.

Investigating the various nucleus states of vSTB showed unique and shared dysregulated gene

expression profiles when comparing eoPE and controls (Figure 4C). We identified 59 (syncytiotrophoblast immature), 289 (syncytiotrophoblast 1), and 412 (syncytiotrophoblast 2) DEGs that were unique for each nucleus state (Figure 4C). The DEG uniquely dysregulated in vSTB2 showed enrichment for lipid biosynthetic processes (GO:0008610), regulation of RNA splicing (GO:0043484), and ubiquitin-mediated proteolysis (hsa04120; Table S12).

Apart from the dysregulated gene profiles unique for each of the 3 vSTB nucleus states, we also found that 29.9% (320 of 1080) of all DEGs in vSTB were shared among states. Moreover, these shared DEGs were congruently upregulated or downregulated across all vSTB nucleus states (96.3%, 308 of 320 genes; Figure 4C). This points to an underlying core dysregulation in the vSTB compartment in eoPE. Dysregulated genes in a concordant direction across vSTB subtypes include targets associated with placental health and dysfunction (Figure 4D).

Importantly, 12.2% of all shared DEG in ≥ 2 vSTB nucleus states were attributed to SASP¹⁶ genes (39/320 genes; 1.94 \times -fold enrichment; hypergeometric $P=6.02 \times 10^{-5}$; Figure 4E; Table S13). We next calculated the community network of statistically significant hub genes to find central genes, such as *FOS* (Figure 4F).

Dysregulation of Trophoblast-Derived SASP Factors in eoPE

Previous studies have shown that 2 SASP factors, activin A²¹ and GDF15,²² are differentially expressed in maternal blood at 36 weeks preceding a diagnosis of preeclampsia. Both factors are secreted proteins

of the transforming growth factor β superfamily, and GDF15 is a marker for vSTB that is secreted into the maternal circulation. Activin A (encoded by *INHBA*) is a known SASP component and also an activin-pathway ligand. We found that in the eoPE cohort, senescence signatures were particularly increased in vSTB1 and vSTB2, which comprise the 2 final nuclear differentiation states within the vSTB (Figure 5A). We confirmed the upregulation of SASP factors, *INHBA*, *GDF15*, and *SERPINE1*, by RT-qPCR in villous tissue from 4 clinical cohorts. The expression was significantly increased in eoPE samples compared with gestational age-matched preterm placental samples (Figure 5B; Figure S9). GDF15 protein was localized to the syncytiotrophoblast cell layer in sections of villi tissue by immunofluorescence staining with no

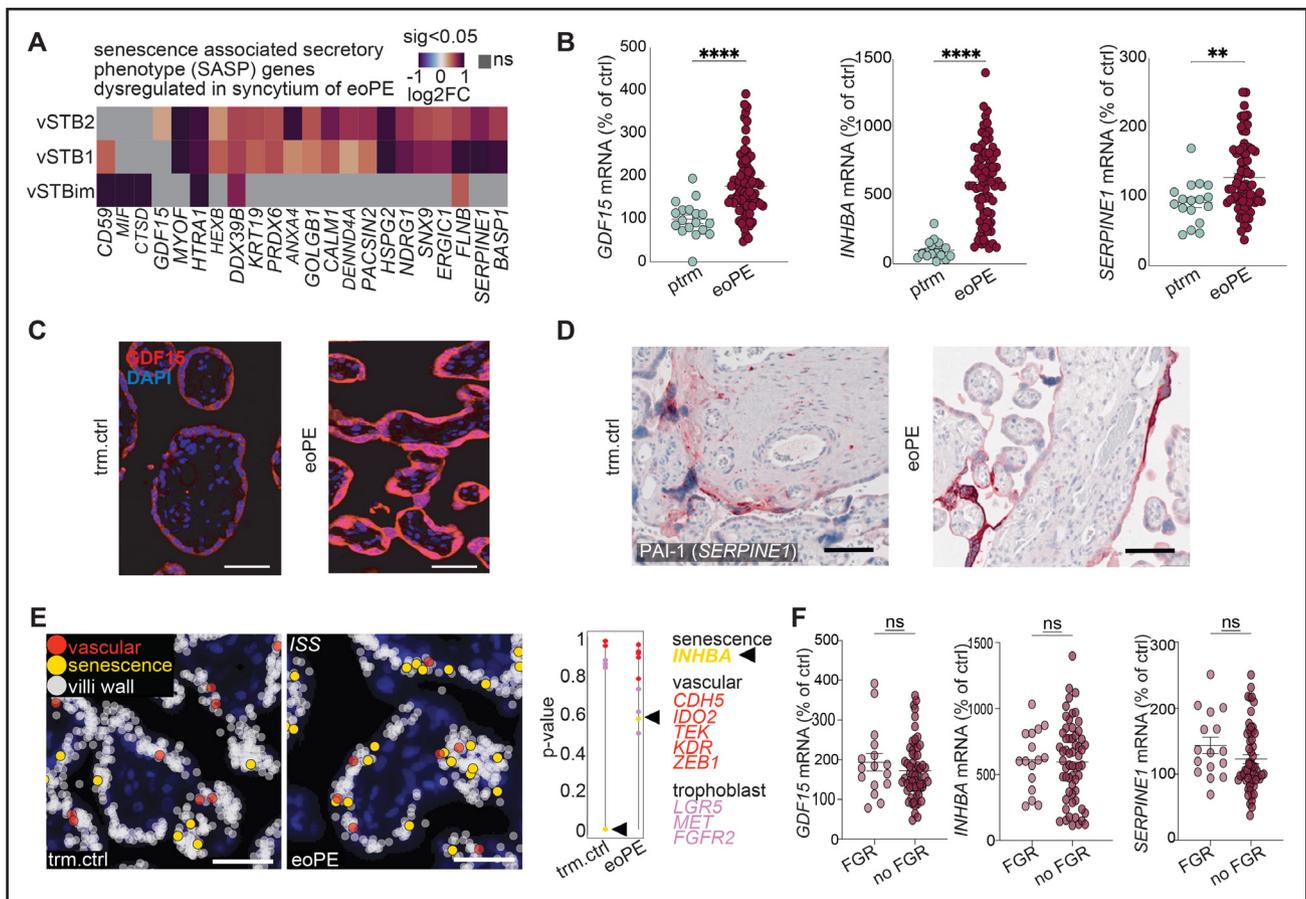


Figure 5. Senescence profile and spatial dysregulation of the maternal-fetal interface in early onset preeclampsia.

A, Heatmap illustrating log₂-fold changes (log₂FC) of senescence-associated secretory phenotype (SASP)-related genes dysregulated in ≥ 2 nuclear villous syncytiotrophoblast (vSTB) states in early onset preeclampsia (eoPE); min. log₂FC 0.4, expressed in $\geq 30\%$ of nuclei and adjusted $P < 0.05$ per gene. **B**, Upregulated mRNA expression (quantitative real-time PCR [RT-qPCR]) of SASP markers *GDF15* (growth differentiation factor 15), *INHBA*, and *SERPINE1* in villous tissue of eoPE in a clinical cohort compared with healthy matched preterm controls (ptm: n=18; eoPE: n=61; sig.level ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; and unpaired 2-tailed Welch *t* test). **C**, Representative immunofluorescence staining of GDF15 in term controls (trm.ctrl) and eoPE (n=3 per group; scale bar, 50 μ m). **D**, Representative immunohistochemical staining of PAI-1 (plasminogen activator inhibitor 1; encoded by the *SERPINE1* gene) in trm.ctrl vs eoPE placentas suggests overall increased expression in the vSTB layer (n=4 per group; scale bar, 40 μ m). **E**, **Left**, Targeted high-resolution spatial transcriptomic data acquired through spatially resolved in situ sequencing (ISS), with spatial context of mRNA molecules implicating highly structured tissue in an unsupervised 2-dimensional embedding (n=2 total, representative area shown; scale bar, 60 μ m). **Right**, Senescence-associated *INHBA* expression is spatially variable in ISS data and is closer to vascular transcripts (black arrows) in eoPE compared with controls. **F**, mRNA expression (RT-qPCR) of SASP markers, *GDF15*, *INHBA*, and *SERPINE1*, is not related to fetal growth restriction (FGR) in eoPE (eoPE without FGR: n=16; eoPE with FGR: n=61; sig.level ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; and unpaired 2-tailed Welch *t* test).

differences between eoPE and healthy term control (Figure 5C). PAI-1 (plasminogen activator inhibitor 1) protein (encoded by *SERPINE1*) similarly confirmed syncytiotrophoblast localization in immunohistochemical analyses (Figure 5D). In addition, we used a high-resolution spatial transcriptomics method (in situ sequencing) to compare the spatial orientation of senescence within the vSTB layer between healthy and eoPE placentas. Our in situ sequencing panel, which detected 174 genes, characterized all villous cell types at high resolution by gene markers identified in our snRNA-seq analyses (Figure 5E; Figure S10). We identified all transcripts associated with the villous surface layer and were able to precisely allocate senescence-associated *INHBA* transcripts to the vSTB (Figure 5E).

To evaluate the interaction between fetal vessels and the trophoblast barrier, we specifically examined fetal vessel markers (*CDH5*, *IDO2*, *KDR*, *TEK*, and *ZEB1*) that were localized within a 5- μ m radius of the villous wall, made up of vCTB and vSTB (Figure S10). Fetal vessels play a direct role in the exchange of nutrients and oxygen between maternal and fetal circulation. In eoPE, senescence-associated *INHBA* expression in the villous trophoblast layer was significantly closer to the fetal vessels compared with term controls (Figure 5E).

This indicates spatial disorganization of the villous wall with increased senescence in areas of transplacental transport. This cannot be explained with a less developed terminal villus compartment in eoPE cases but points to a major change in the morphological and functional organization of such villi. Aberrant senescence in this vital maternal-fetal interphase might interfere with the exchange of nutrients and metabolites, potentially resulting in FGR that is often associated with eoPE. However, we found no differences in the expression of SASP targets, *INHBA*, *GDF15*, and *SERPINE1*, between pregnancies with or without FGR (Figure 5F). This indicates that the observed SASP phenotype is indeed eoPE-specific.

Syncytiotrophoblast Senescence and SASP Factor Secretion in eoPE

We next investigated whether eoPE senescence localized in the vSTB layer may result in the secretion of SASP factors into the maternal circulation (Figure 6). In search of receptor-ligand interactions that may represent fetal to maternal signaling via secreted SASP proteins, we examined our snRNA-seq data from the decidua, which included the signature of maternal vessels. We analyzed

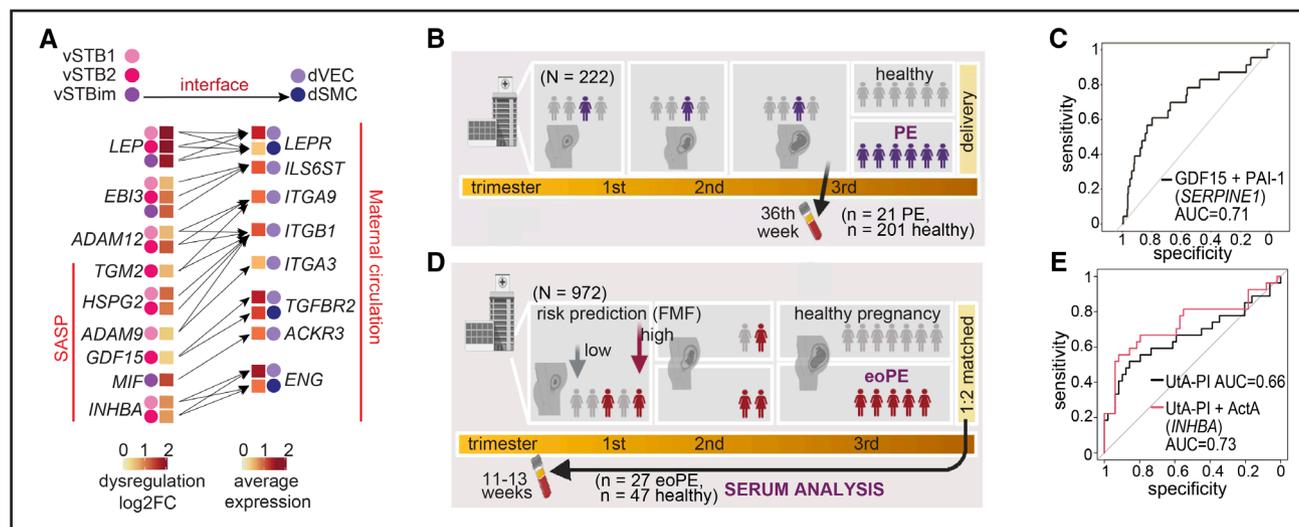


Figure 6. Senescence factors derived from the maternal-fetal interface are detectable in maternal blood and serve as good predictors for preeclampsia (PE).

A, Dysregulated and secreted ligands from the villous syncytiotrophoblast (vSTB) in early onset PE (eoPE) act on highly expressed receptors in decidual vascular endothelial cells (vVECs) and smooth muscle cells (dSMCs). This highlights ligand pressure, that is, increased ligand expression with unaltered receptor expression at the maternal-fetal interface. Arrows point toward receptors (Wilcoxon rank-sum test, $P < 0.05$, identified by multiple tools and databases). Dot colors encode cell types/states, ligand squares represent upregulated secreted log₂-fold change (log₂FC) expression per nuclear state, and receptor squares encode average expression. Senescence-associated secretory phenotypes (SASPs) are marked. **B**, Schematic illustrating the maternal blood sampling strategy at 36 weeks of pregnancy before diagnosis of PE. Samples are part of the large-scale Australian Triple B Study: Bumps, Babies and Beyond (BUMPS) cohort (n=222). **C**, Circulating GDF15 (growth differentiation factor 15) and PAI-1 (plasminogen activator inhibitor 1; encoded by *SERPINE1*) are characteristic for the preeclamptic syndrome and are used as predictive markers for PE (n=21; healthy term controls: n=201). **D**, Schematic illustrating published maternal first-trimester serum sampling strategy of prospectively recruited women healthy term, and eoPE pregnancies were diagnosed in later gestation (n=972). **E**, Circulating activin A, synthesized by inhibin β A dimers (encoded by *INHBA*), predicts eoPE in a conditional logistic regression model and shows that the currently used Fetal Maternal Foundation (FMF) algorithm underestimates eoPE risk in women with high first-trimester activin A (eoPE: n=27; healthy term controls: n=47, matched for body mass index, gestational age, and maternal age; subset of cohort is shown in **D**). AUC indicates area under the curve.

Downloaded from <http://ahajournals.org> by on April 23, 2025

factors that are secreted by vSTBim, vSTB1, and vSTB2 and were differentially expressed in eoPE compared with healthy term controls (Figure 6A; Table S11). Indeed, we found that secreted factors, such as LEP (leptin) or TGM2 (transglutaminase 2) and HSPG2 (heparan sulfate proteoglycan 2), which were upregulated in eoPE, interacted with receptors expressed on vascular cells (decidual vascular endothelial cells and decidual smooth muscle cells) of the decidua ($\log_2FC > 0.25$, cutoff: genes detected in a minimum of 10% of diseased cells, Benjamini-Hochberg false discovery rate-corrected $P < 0.01$; Figure 6A). Of note, the interactions between vSTB-decidual vascular endothelial cells and vSTB-decidual smooth muscle cells predominantly involved SASP genes (67%, 6 of 9), including *INHBA* and *GDF15*.

To validate the increased syncytiotrophoblast senescence and evaluate the predictive potential and pathogenic relevance of our SASP markers, we analyzed maternal blood from 2 cohorts (Figures 6B and 6D; see detailed statistics in the Supplemental Methods), including eoPE or late-onset preeclampsia. The first cohort comprised the Triple B Study: Bumps, Babies and Beyond (BUMPS; Figure 6B and 6C).²³ We performed a nested case-cohort study where from a pool of 1000 samples, 23 samples ($n=23$) were selected, which subsequently developed preeclampsia and were compared with 199 randomly selected control samples ($n=199$; Figure 6B; Table S1). Univariate logistic regression models showed that elevated blood levels of PAI-1 (mean control 40 767 pg/mL versus preeclampsia 48 624 pg/mL) and GDF15 (mean control 84 281 pg/mL versus preeclampsia 118 694 pg/mL) at 36 weeks gestation were able to predict the later development of preeclampsia (area under the curve, 0.64; $P=0.023$; area under the curve, 0.70; $P=0.002$, respectively; Figure 6C). Furthermore, multivariate logistic regression models showed that these effects persisted after adjustment for body mass index and nulliparity (PAI-1; adjusted odds ratio, 1.43 per 10 ng/mL [95% CI, 1.06–1.95]; $P=0.021$; GDF15: adjusted odds ratio, 1.17 per 10 ng/mL [95% CI, 1.06–1.31]; $P=0.003$). These data suggest that the observed increase in syncytiotrophoblast senescence may be a common feature of preeclampsia independent of gestational age and underlying clinical risk factors.

We next measured circulating levels of the SASP factor activin A in a second cohort in which blood samples were obtained during the first trimester of pregnancy (11–13 weeks; Figure 6D). We selected a case-control matched 1:2 (27 cases of eoPE and 47 controls after the technical exclusion of 5 samples; Figure 6D; Table S1). Women with comorbidities, such as chronic hypertension or diabetes, were excluded, and conditional logistic regression modeling was used. Activin A (encoded by the gene *INHBA*) contributes to eoPE prediction already in early pregnancy. Elevated resistance in the uterine artery in the first trimester (measured via the

uterine artery pulsatility index [UtA-PI] measurements) is associated with an increased risk of eoPE. Receiver operating characteristic curve analysis of UtA-PI in combination with activin A had a higher area under the curve compared with UtA-PI alone in the case-control samples (Figure 6E). Our conditional logistic regression model adjusted for the Fetal Medicine Foundation risk assessment showed high circulating levels of activin A and was significantly associated with the development of eoPE (adjusted odds ratio, 2.17 per $\mu\text{g/mL}$ [95% CI, 1.03–4.58]; $P=0.042$).

DISCUSSION

The pathogenesis of preeclampsia is still not fully understood, but there is compelling evidence to suggest that it is initiated in early pregnancy. This study combines, for the first time, a variety of cohorts, techniques, and modeling algorithms, resulting in a novel view of eoPE pathophysiology. It complements recent studies that have characterized primarily the healthy human maternal-fetal interphase at the single-cell level.^{9–15} However, in contrast to scRNA-seq mainly applied in these studies, the use of snRNA-seq allowed us to characterize, for the first time, the rich nuclear diversity of the abundant syncytiotrophoblast syncytium in direct contact with maternal blood. Here, transcriptomic profiling by snRNA-seq preserves a true cytotrophoblast/syncytiotrophoblast trophoblast ratio of 1:9, whereby, in scRNA-seq, the ratio is nearly inverted.^{10,11} The main results of our study indicate that eoPE is largely based on dysregulation of the multinucleated syncytiotrophoblast due to increased senescence. Within the multinucleated syncytiotrophoblast, the 3 nuclei states, syncytiotrophoblast immature, syncytiotrophoblast 1, and syncytiotrophoblast 2, are already dysregulated in the first trimester, resulting in enhanced expression and secretion of SASP factors, such as activin A and GDF15. Of note, we show that levels of these factors released into the maternal circulation can predict pregnancies destined to develop preeclampsia. Indeed, in situ sequencing results demonstrate increased proximity of *INHBA* (coding for activin A) to fetal vessels in eoPE tissue compared with controls. Thus, early and continuous syncytiotrophoblast secretion of SASP factors into the maternal circulation may largely contribute to the placental defect and maternal syndromes, which are characteristics of eoPE.

As fetal EVT invasion into the maternal decidua is essential, it was previously hypothesized that faulty spiral artery remodeling by EVT causes preeclampsia.^{3,24} Due to the naturally low abundance of CTT/EVT, enrichment of HLA-G^{pos} cells to obtain a higher yield is commonly used to study invasion.¹⁰ A limitation of this study is the lack of comparative analyses in these groups due to too few cell column trophoblast/EVTs recovered from the decidua samples by vacuum suctioning, given our lack

of cell population enrichment. The association between EVT invasion and an enhanced UtA-PI of the uterine artery preceding decidual spiral arteries is debated.^{25,26} However, in eoPE, the UtA-PI has a moderate predictive sensitivity of 12% to 61.5%²⁷ and low predictive potential for term preeclampsia²⁷ yet a high sensitivity for FGR.^{27,28} This indicates that preeclampsia development is most likely linked not only to faulty uterine artery remodeling and flow but also, as previously described, to multifactorial causes,^{29,30} including altered syncytiotrophoblast maturation and functioning,³¹ as well as increased syncytiotrophoblast senescence. Altered senescence profiles may indeed be a major aspect of all preeclampsia subvariants, regardless of their multifactorial origin.^{29,30}

The problem of classifying preeclampsia correctly and acknowledging that there is not just 1 causative factor poses a core problem for understanding eoPE pathogenesis.²⁹ In addition, little is known about eoPE pathogenesis in the first trimester of pregnancy and before the onset of clinical symptoms. Our gene expression data suggest that aberrant syncytiotrophoblast senescence may indeed be a key factor involved in eoPE development. However, the molecular pathways that would result in increased SASP formation within the syncytiotrophoblast layer remain unknown and need to be tackled in the future.

Potential causes for syncytiotrophoblast-associated alterations that lead to SASP secretion into maternal blood may be premature trophoblast differentiation and mitochondrial stress, as recently shown in mice using designer receptors exclusively activated by designer drug techniques.³² Thus, their inclusion in panels of known anthropometric and biochemical predictors of preeclampsia in early pregnancy, such as co-peptin,³³ could improve clinical diagnostic accuracy in the first trimester. Importantly, it would be of future interest to assess how SASP factors, such as activin A, PAI-1, and GDF15, induce syncytiotrophoblast damage and whether these mechanisms of action can be therapeutically targeted to improve clinical management.

Overall, various early triggers^{29,30} and known clinical preeclampsia risk factors may converge in an accelerated turnover of the villous trophoblast that ultimately culminates in maternal-fetal barrier dysfunction. This accelerated turnover may, in turn, induce premature syncytiotrophoblast senescence, as observed in the maternal circulation of early onset and late-onset preeclampsia cohorts.³² Therefore, SASP factor upregulation in the syncytiotrophoblast of eoPE compared with preterm controls validates that this mechanism is eoPE-specific. These findings align with observations of cardiovascular disease beyond pregnancy in which SASP factors are known to play a role.^{33–35} Nevertheless, the choice of healthy term control samples poses a limitation of this study, as we chose to profile healthy term controls as a physiological baseline for the end of

pregnancy and integrated scRNA-seq and microarray data to computationally correct for a potential preterm effect. It is not ethical to perform serial placental biopsies across pregnancy without a clinical implication. It is, thus, impossible to determine whether early pregnancy placenta samples are from pregnancies destined to develop preeclampsia.² Yet, characterization of the pathophysiology of preeclampsia at early gestational stages would be necessary to allow novel biological insights, which could improve clinical management.^{1,2} Our results strongly suggest that SASP factors appear to be such key mediators of placental tissue dysregulation seen in eoPE.

We envision that our comprehensive multiomics study will lead to the discovery of further important pathophysiological mechanisms, resulting in the generation of eoPE. It may eventually lead to novel treatments including those that target SASP factors. Ultimately, the generation of new pharmacological treatments to prevent preeclampsia that would already be initiated in early pregnancy may also help to decrease the development of cardiovascular disease in post-preeclampsia mothers and their children in the long term.

PERSPECTIVES

There is a prevailing unmet need to develop novel therapeutics to treat the life-threatening and prevalent obstetric disease preeclampsia to protect mother and child in pregnancy and their increased risk of cardiovascular disease later in life. This depends largely on our understanding of early molecular mechanisms that drive preeclampsia pathophysiology, which has remained largely unknown. Here, we profiled the different transcriptomic states of the maternal-fetal interface at the single-cell level in early and late gestation. In eoPE, vSTB nuclei are associated with an increased SASP profile, including important markers activin A (encoded by *INHBA* gene) and PAI-1 (encoded by *SERPINE1*). We found that circulating levels of these factors act as predictive markers for eoPE and preeclampsia before clinical symptoms or diagnosis. The premature vSTB senescence and predictive power before symptom onset suggest that SASP is one of the mediators of placental dysregulation seen in eoPE pathogenesis. Targeting the SASP pathway in early pregnancy may prevent preeclampsia development and its associated life-long cardiovascular complications. Thus, integrating these findings into clinical practice is expected to revolutionize prenatal care by enabling personalized monitoring and intervention strategies for at-risk women. Furthermore, more advanced studies of maternal-fetal interactions in preeclampsia will lead to a more comprehensive understanding of pregnancy health and disease, ultimately improving outcomes for mother and child in both short and long terms.

ARTICLE INFORMATION

Received May 22, 2024; accepted October 7, 2024.

Affiliations

Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany (O.N., D.S.V., J.U., H.B., A.F., N.H., K.K., S.K., D.N.M., R.D., F.H.). Experimental and Clinical Research Center, a cooperation between the Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association and the Charité – Universitätsmedizin Berlin, Germany (O.N., D.S.V., J.U., H.B., A.F., N.H., K.K., S.K., R.S.-U., D.N.M., R.D., F.H.). Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany (O.N., D.S.V., J.U., H.B., A.F., N.H., K.K., S.K., R.S.-U., F.C., D.N.M., R.D., F.H.). German Center for Cardiovascular Research (DZHK), Berlin, Germany (O.N., D.S.V., H.B., N.H., K.K., S.K., D.N.M., R.D.). Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center (O.N., K.S., T. Kroneis, L.N., D.F., B.H., A.E.-H., M.G.) and Institute for Medical Informatics, Statistics and Documentation (T. Kuenzer), Medical University of Graz, Austria. Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Center of Digital Health, Germany (O.D., S. Tiesmeyer, P.K., A.M., N.M.-B., R.E., N.J.). Centre for Biomarker Research in Medicine, Graz, Austria (K.S., T. Kroneis, A.E.-H.). Max Delbrück Center - Berlin Institute for Medical Systems Biology (MDC-BIMSB), Berlin, Germany (A.K.S., C.F.). Department of Biology, Chemistry and Pharmacy, Institute of Chemistry and Biochemistry, Freie Universität Berlin, Germany (A.K.S.). Department of Obstetrics and Gynaecology, Reproductive Biology Unit, Medical University of Vienna, Austria (T.M., M.K., S.H.). Institute of Molecular Biology and Genetic of the National Academy of Sciences of Ukraine (NASU), Kyiv, Ukraine (A.F.). Department of Obstetrics and Gynaecology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany (S.V.). Department of Obstetrics and Gynaecology, University Hospital Graz, Medical University Graz, Austria (C.S.). Institute for Clinical Medicine, Faculty of Medicine, University of Oslo, Norway (M.S., A.C.S.). Division of Obstetrics and Gynaecology, Oslo University Hospital, Norway (M.S., A.C.S.). Clinical Biochemistry, King George's Hospital, London, United Kingdom (S.J.). Fetal Medicine Unit, St George's University Hospitals NHS Foundation Trust, London, United Kingdom (B.T.). Department of Obstetrics and Gynaecology (Mercy Hospital for Women), The University of Melbourne, VIC, Australia (T.J.K.-L., S. Tong). Department of Cardiology and Nephrology, HELIOS Clinic, Berlin, Germany (R.D.).

Acknowledgments

The authors gratefully appreciate the excellent technical assistance of May-Britt Köhler, Gabriele N'Diaye, Jana Czychi, Kornelia Buttke (Experimental and Clinical Research Centre [ECRC], Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, and Charité), Sabine Maninger (Medical University of Graz), and Ping Cannon, Tuong-Vi Nguyen, and Anna Nguyen (Melbourne, Australia). They also thank Dr Andreas Glasner (Femina-Med) for the patient recruitment of first-trimester placental material. They also thank research nurse Bettina Amtmann (Medical University of Graz) and Gabrielle Pell and team (Melbourne) for the collection of placental tissue and Danica Idzes and Alison Abboud for collection of blood samples in Melbourne. They also thank Daniel Kummer for the image analysis. Schematic drawings were created with biorender.com.

Sources of Funding

D.N. Müller and F. Herse were supported by Deutsche Forschungsgemeinschaft (grants HE6249/5-1, HE6249/7-1, and HE6249/7-2; D.N. Müller: project number 394046635-SFB 1365) and the BIH Omics Platform. F. Herse was funded by VolkswagenStiftung (grant 9D289). D.N. Müller was supported by grants from the German Centre for Cardiovascular Research (DZHK; BER 1.1 VD). O. Nonn was supported through the PhD Program Inflammatory Disorders in Pregnancy (DP-IDP) by the Austrian Science Fund (FWF): Doc 31-B26, PhD Program Molecular Medicine at the Medical University of Graz, Marietta Blau Grant (Austrian Agency for Education and Internationalisation [OeAD]); Austrian Federal Ministry of Education, Science and Research [BMBWF], and grants from the MeFo Graz (PS-Stipendium 2019/2020), German Association of Prenatal Diagnostics and Obstetrics (DGPGM, 2020). K. Sallinger, T. Kuenzer, and A. El-Heliebi were supported by the K1 Competence Centers for Excellent Technologies (COMET), Center for Biomarker Research in Medicine, funded by the BMVIT, Land Steiermark (Department 12, Business and Innovation), BMWFW, the Styrian Business Promotion Agency, and the Vienna Business Agency. The COMET Program is executed by the Austrian Research Promotion Agency (FFG). K. Sallinger was supported by the Doctoral School in Translational Molecular and Cellular Biosciences at the Medical University of Graz. S. Haider and T. Maxian (grant P34588), M. Knöfler (grant P31470-B30), and M. Gauster (grants 10.55776/P33554, 10.55776/P35118, 10.55776/DOC31, and 10.55776/I6907) were supported by FWF. B. Huppertz was supported by the FWF: Doc 31-B26 and the Medical University Graz through DP-IDP. F. Coscia and J. Nimo were supported by the German Federal Ministry of

Education and Research (BMBF), as part of MSCoreSys (grant 161L0222). S. Tiesmeyer was supported by BMBF in the framework of Societal Aspects of Genomics (SAGE; grant 031L0265). S. Tong and T.J. Kaitu'u-Lino were supported by the National Health and Medical Research Council of Australia (grants 1159261, 1136418, 1065854, and 2000732). This work was supported by the BMBF-funded German Network for Bioinformatics Infrastructure (de.NBI).

Disclosures

None.

Supplemental Material

Supplemental Materials and Methods

Tables S1–S13

Figures S1–S10

References 36–47

REFERENCES

- Say L, Chou D, Gemmill A, Tuncalp O, Moller AB, Daniels J, Gulmezoglu AM, Temmerman M, Alkema L. Global causes of maternal death: a WHO systematic analysis. *Lancet Glob Health*. 2014;2:e323–e333. doi: 10.1016/S2214-109X(14)70227-X
- Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Adoyi G, Ishaku S; International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. *Hypertension*. 2018;72:24–43. doi: 10.1161/HYPERTENSIONAHA.117.10803
- Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. *Circ Res*. 2019;124:1094–1112. doi: 10.1161/CIRCRESAHA.118.313276
- Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. *Lancet*. 2006;367:1066–1074. doi: 10.1016/S0140-6736(06)68397-9
- Williams D. Long-term complications of preeclampsia. *Semin Nephrol*. 2011;31:111–122. doi: 10.1016/j.semnephrol.2010.10.010
- Brouwers L, van der Meiden-van Roest AJ, Savelkoul C, Vogelvang TE, Lely AT, Franx A, van Rijn BB. Recurrence of pre-eclampsia and the risk of future hypertension and cardiovascular disease: a systematic review and meta-analysis. *BJOG*. 2018;125:1642–1654. doi: 10.1111/1471-0528.15394
- Staff AC. The two-stage placental model of preeclampsia: an update. *J Reprod Immunol*. 2019;134:135:1–10. doi: 10.1016/j.jri.2019.07.004
- Huppertz B, Sammar M, Chefetz I, Neumaier-Wagner P, Bartz C, Meiri H. Longitudinal determination of serum placental protein 13 during development of preeclampsia. *Fetal Diagn Ther*. 2008;24:230–236. doi: 10.1159/000151344
- Suryawanshi H, Morozov P, Straus A, Sahasrabudhe N, Max KEA, Garzia A, Kustagi M, Tuschl T, Williams Z. A single-cell survey of the human first-trimester placenta and decidua. *Sci Adv*. 2018;4:eaa4788. doi: 10.1126/sciadv.aau4788
- Vento-Tormo R, Eremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, Park JE, Stephenson E, Polanski K, Goncalves A, et al. Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature*. 2018;563:347–353. doi: 10.1038/s41586-018-0698-6
- Pique-Regi R, Romero R, Tarca AL, Sendler ED, Xu Y, Garcia-Flores V, Leng Y, Luca F, Hassan SS, Gomez-Lopez N. Single cell transcriptional signatures of the human placenta in term and preterm parturition. *Elife*. 2019;8:e52004. doi: 10.7554/eLife.52004
- Arutyunyan A, Roberts K, Troule K, Wong FCK, Sheridan MA, Kats I, Garcia-Alonso L, Veltan B, Hoo R, Ruiz-Morales ER, et al. Spatial multiomics map of trophoblast development in early pregnancy. *Nature*. 2023;616:143–151. doi: 10.1038/s41586-023-05869-0
- Liu Y, Fan X, Wang R, Lu X, Dang YL, Wang H, Lin HY, Zhu C, Ge H, Cross JC, et al. Single-cell RNA-seq reveals the diversity of trophoblast subtypes and patterns of differentiation in the human placenta. *Cell Res*. 2018;28:819–832. doi: 10.1038/s41422-018-0066-y
- Wang M, Liu Y, Sun R, Liu F, Li J, Yan L, Zhang J, Xie X, Li D, Wang Y, et al. Single-nucleus multi-omic profiling of human placental syncytiotrophoblasts identifies cellular trajectories during pregnancy. *Nat Genet*. 2024;56:294–305. doi: 10.1038/s41588-023-01647-w
- Garcia-Alonso L, Handfield LF, Roberts K, Nikolakopoulou K, Fernando RC, Gardner L, Woodhams B, Arutyunyan A, Polanski K, Hoo R, et al. Mapping the temporal and spatial dynamics of the human endometrium in vivo and in vitro. *Nat Genet*. 2021;53:1698–1711. doi: 10.1038/s41588-021-00972-2

16. Basisty N, Kale A, Jeon OH, Kuehnemann C, Payne T, Rao C, Holtz A, Shah S, Sharma V, Ferrucci L, et al. A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS Biol.* 2020;18:e3000599. doi: 10.1371/journal.pbio.3000599
17. Leavey K, Bainbridge SA, Cox BJ. Large scale aggregate microarray analysis reveals three distinct molecular subclasses of human preeclampsia. *PLoS One.* 2015;10:e0116508. doi: 10.1371/journal.pone.0116508
18. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet.* 2008;371:75–84. doi: 10.1016/S0140-6736(08)60074-4
19. Baergen RN, Burton GJ, Kaplan CG. *Benirschke's Pathology of the Human Placenta.* 7 ed. Springer Nature; 2023.
20. Huppertz B, Frank HG, Kingdom JC, Reister F, Kaufmann P. Villous cytotrophoblast regulation of the syncytial apoptotic cascade in the human placenta. *Histochem Cell Biol.* 1998;110:495–508. doi: 10.1007/s004180050311
21. Muttukrishna S, Knight PG, Groome NP, Redman CW, Ledger WL. Activin A and inhibin A as possible endocrine markers for pre-eclampsia. *Lancet.* 1997;349:1285–1288. doi: 10.1016/s0140-6736(96)09264-1
22. Cruickshank T, MacDonald TM, Walker SP, Keenan E, Dane K, Middleton A, Kyritsis V, Myers J, Cluver C, Hastie R, et al. Circulating growth differentiation factor 15 is increased preceding preeclampsia diagnosis: implications as a disease biomarker. *J Am Heart Assoc.* 2021;10:e020302. doi: 10.1161/JAHA.120.020302
23. Kaitu'u-Lino TJ, MacDonald TM, Cannon P, Nguyen TV, Hiscock RJ, Haan N, Myers JE, Hastie R, Dane KM, Middleton AL, et al. Circulating SPINT1 is a biomarker of pregnancies with poor placental function and fetal growth restriction. *Nat Commun.* 2020;11:2411. doi: 10.1038/s41467-020-16346-x
24. Brosens I, Pijnenborg R, Vercruyse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol.* 2011;204:193–201. doi: 10.1016/j.ajog.2010.08.009
25. Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. *Hypertension.* 2013;62:1046–1054. doi: 10.1161/HYPERTENSIONAHA.113.01892
26. James-Allan LB, Whitley GS, Leslie K, Wallace AE, Cartwright JE. Decidual cell regulation of trophoblast is altered in pregnancies at risk of pre-eclampsia. *J Mol Endocrinol.* 2018;60:239–246. doi: 10.1530/JME-17-0243
27. Tian Y, Yang X. A review of roles of uterine artery Doppler in pregnancy complications. *Front Med (Lausanne).* 2022;9:813343. doi: 10.3389/fmed.2022.813343
28. Pilalis A, Souka AP, Antsaklis P, Basayiannis K, Benardis P, Haidopoulos D, Papantoniou N, Mesogitis S, Antsaklis A. Screening for pre-eclampsia and small for gestational age fetuses at the 11-14 weeks scan by uterine artery Dopplers. *Acta Obstet Gynecol Scand.* 2007;86:530–534. doi: 10.1080/00016340601155056
29. Roberts JM, Rich-Edwards JW, McElrath TF, Garmire L, Myatt L; Global Pregnancy Collaboration. Subtypes of preeclampsia: recognition and determining clinical usefulness. *Hypertension.* 2021;77:1430–1441. doi: 10.1161/HYPERTENSIONAHA.120.14781
30. Robillard PY, Dekker G, Scioscia M, Bonsante F, Iacobelli S, Boukerrou M, Hulsey TC. The blurring boundaries between placental and maternal preeclampsia: a critical appraisal of 1800 consecutive preeclamptic cases. *J Matern Fetal Neonatal Med.* 2022;35:2450–2456. doi: 10.1080/14767058.2020.1786516
31. Huppertz B. Trophoblast differentiation, fetal growth restriction and preeclampsia. *Pregnancy Hypertens.* 2011;1:79–86. doi: 10.1016/j.preghy.2010.10.003
32. Opichka MA, Livergood MC, Balapattabi K, Ritter ML, Brozowski DT, Wackman KK, Lu KT, Kozak KN, Wells C, Fogo AB, et al. Mitochondrial-targeted antioxidant attenuates preeclampsia-like phenotypes induced by syncytiotrophoblast-specific Galphaq signaling. *Sci Adv.* 2023;9:eadg8118. doi: 10.1126/sciadv.adg8118
33. Santillan MK, Santillan DA, Scroggins SM, Min JY, Sandgren JA, Pearson NA, Leslie KK, Hunter SK, Zamba GK, Gibson-Corley KN, et al. Vasopressin in preeclampsia: a novel very early human pregnancy biomarker and clinically relevant mouse model. *Hypertension.* 2014;64:852–859. doi: 10.1161/hypertensionaha.114.03848
34. Ali I, Zhang H, Zaidi SAA, Zhou G. Understanding the intricacies of cellular senescence in atherosclerosis: mechanisms and therapeutic implications. *Ageing Res Rev.* 2024;96:102273. doi: 10.1016/j.arr.2024.102273
35. Roh JD, Castro C, Yu A, Rana S, Shahul S, Gray KJ, Honigberg MC, Ricke-Hoch M, Iwamoto Y, Yeri A, et al; IPAC Investigators. Placental senescence pathophysiology is shared between peripartum cardiomyopathy and preeclampsia in mouse and human. *Sci Transl Med.* 2024;16:eadi0077. doi: 10.1126/scitranslmed.adi0077
36. Nyarady BB, Kiss LZ, Bagyura Z, Merkely B, Dosa E, Lang O, Kohidai L, Pallinger E. Growth and differentiation factor-15: a link between inflammaging and cardiovascular disease. *Biomed Pharmacother.* 2024;174:116475. doi: 10.1016/j.biopha.2024.116475
37. Harsem NK, Staff AC, He L, Roald B. The decidual suction method: a new way of collecting decidual tissue for functional and morphological studies. *Acta Obstet Gynecol Scand.* 2004;83:724–730. doi: 10.1080/j.0001-6349.2004.00395.x
38. Krishnaswami SR, Grindberg RV, Novotny M, Venepally P, Lacar B, Bhutani K, Linker SB, Pham S, Erwin JA, Miller JA, et al. Using single nuclei for RNA-seq to capture the transcriptome of postmortem neurons. *Nat Protoc.* 2016;11:499–524. doi: 10.1038/nprot.2016.015
39. Slyper M, Porter CBM, Ashenberg O, Waldman J, Drokhyansky E, Wakiro I, Smillie C, Smith-Rosario G, Wu J, Dionne D, et al. A single-cell and single-nucleus RNA-seq toolbox for fresh and frozen human tumors. *Nat Med.* 2020;26:792–802. doi: 10.1038/s41591-020-0844-1
40. Luecken MD, Buttner M, Chaichoompu K, Danese A, Interlandi M, Mueller MF, Strobl DC, Zappia L, Dugas M, Colome-Tatche M, et al. Benchmarking atlas-level data integration in single-cell genomics. *Nat Methods.* 2022;19:41–50. doi: 10.1038/s41592-021-01336-8
41. Boyeau P, Lopez R, Regier J, Gayoso A, Jordan MI, Yosef N. Deep generative models for detecting differential expression in single cells. *bioRxiv.* Preprint posted online October 4, 2019. doi: 10.1101/794289
42. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 2015;43:e47. doi: 10.1093/nar/gkv007
43. Dimitrov D, Turei D, Garrido-Rodriguez M, Burmedi PL, Nagai JS, Boys C, Ramirez Flores RO, Kim H, Szalai B, Costa IG, et al. Comparison of methods and resources for cell-cell communication inference from single-cell RNA-seq data. *Nat Commun.* 2022;13:3224. doi: 10.1038/s41467-022-30755-0
44. Guy GP, Leslie K, Diaz Gomez D, Forenc K, Buck E, Khalil A, Thilaganathan B. Implementation of routine first trimester combined screening for preeclampsia: a clinical effectiveness study. *BJOG.* 2021;128:149–156. doi: 10.1111/1471-0528.16361
45. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, Carbone IF, Dutemeyer V, Fiolna M, Frick A, et al. Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations. *Ultrasound Obstet Gynecol.* 2017;49:756–760. doi: 10.1002/uog.17455
46. Xu H, Qian J, Paynter NP, Zhang X, Whitcomb BW, Tworoger SS, Rexrode KM, Hankinson SE, Balasubramanian R. Estimating the receiver operating characteristic curve in matched case control studies. *Stat Med.* 2019;38:437–451. doi: 10.1002/sim.7986
47. Bartho LA, Kandel M, Walker SP, Cluver CA, Hastie R, Bergman L, Pritchard N, Cannon P, Nguyen TV, Wong GP, et al. Circulating chemerin is elevated in women with preeclampsia. *Endocrinology.* 2023;164:bqad041. doi: 10.1210/endo/bqad041