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By: CHARIK Amira

TOPIC

Biochemical and molecular foundations of preeclampsia and their Impact on offspring cardiovascular risk

Jury:

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Dedication

I dedicate this research to the first person who held my little hand and pulled it towards the seats of knowledge and learning, to the one who overwhelmed me with his love and affection, to the one who pushed me to take the very first step to face life and said that he is with me, to ***my dear grandfather***. So from this point that I have reached now I say that I love you, you who drew for me the starting line and said "set off."

To ***my mother*** and ***father***, my grandmother and my siblings ***Aya***, ***Ferial***, ***Salsabil***, ***Mohamed***, and ***Bilal***, to my beloved ones and the flowers of the garden of my heart, which is scented with the fragrance of their love, their support, and their standing by me, with a little of the fragrance of playful madness, I love you my little treasure.

And to my big family, to ***my uncles*** and ***aunts***, maternal and paternal, thank you for your support, your love, and your care.

To the two people who believed in me the most, to my two dear professors ***Bakour Hamza*** and ***Kasmi Hamza***, what a coincidence, so I am not surprised that I love two people who not only share the same name but also shared in supporting me, trusted me, and stood by me until the last point. So I greet them and I pray to God to show them the most beautiful things of this world and to keep all harm away from them.

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To my life companion and half of my soul, my beloved of my heart, ***Kawthar***, thank you for everything, I love you my crazy one. To my friend ***Sarra***, who stood with me in the most difficult stages of life and bestowed upon me from her wisdom and soundness of mind to plant hope in me every time. To my beloved sister whom I never saw as just a friend, ***Rania***, and to her kind family who always send prayers of success and prosperity to a girl they considered as their own daughter, thank you so much for everything. And to my dear friend ***Mounia***, the kind girl with innocent and childish features, thank you for your love and support. And to the companion of my soul and my support, my beloved ***Khawla***, thank you for everything, O owner of the kind heart. To everyone who stayed by my side even from a distant place, watching over me and praying for me from the depths of their heart — and to every person whose name I forgot to mention, I say that I am sorry; please know that I have always appreciated and will continue to appreciate your efforts and support. And in the end, to ***myself*** who never gave up and never despaired, to the first warrior in the battle of my life.

So, praise and thanks be to God always and forever for everything, for every blessing and every hardship, for everything is by His wisdom and His power.

Thank you

First and foremost, I thank Almighty God for granting me the health, patience, and determination to complete this dissertation.

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Summary

ملخص

يُعَدّ تسمم الحمل اضطراباً حملياً معقداً يتميز بارتفاع ضغط الدم وينشأ نتيجة اختلالات بيوكيميائية وجزيئية على مستوى الواجهة المشيمية—الأمومية. وتشمل الآليات المركزية الإجهاد التأكسدي، والخلل البطاني، واختلال التوازن في العوامل الوعائية، حيث يتمثل ذلك في تغير مستويات VEGF و PlGFو .sFlt-1كما يُسهم اضطراب نظام الرينين—أنجيوتنسين—ألدوستيرون، وزيادة إنتاج أنواع الأكسجين التفاعلية، وارتفاع الوسائط الالتهابية مثل-VCAM 1في تفاقم الضرر الوعائي وانخفاض توافر أوكسيد النيتريك. تؤدي هذه العمليات الفيزيوباثولوجية إلى نقص التأكسج المشيمي وتنشيط بطاني جهازي، وهو ما يفسر السمات السريرية لتسمم الحمل. والأهم من ذلك، أن البيئة الرحمية غير المواتية تترك آثاراً طويلة الأمد على النسل؛ إذ يرتبط نقص أوكسيد النيتريك في دم الحبل السري، والتغيرات في التعبير الجيني للجزيئات اللاصقة، والتعديلات فوق الجينية بزيادة القابلية للإصابة المبكرة بالاختلال الوعائي وارتفاع خطر الأمراض القلبية الوعائية لاحقاً. وعليه، يُمثل تسمم الحمل ليس فقط مضاعفة حمليّة بل محدداً أساسياً للصحة القلبية الوعائية عبر الأجيال، مما يبرز الحاجة إلى استراتيجيات موجهة للتقليل من عواقبه طويلة المدى على نسل الأمهات المصابات.

الكلمات المفتاحية : تسمم الحمل ، الإجهاد التأكسدي ، الخلل البطاني، المخاطر القلبية الوعائية ، التعديلات فوق الجينية.

Résumé

La prééclampsie est un trouble hypertensif complexe de la grossesse, résultant de perturbations biochimiques et moléculaires à l'interface materno-placentaire. Les mécanismes centraux incluent le stress oxydatif, la dysfonction endothéliale et le déséquilibre angiogénique, caractérisé par des niveaux altérés de VEGF, PlGF et sFlt-1. La dérégulation du système rénine–angiotensine–aldostérone, l'augmentation de la production d'espèces réactives de l'oxygène et la libération accrue de médiateurs pro-inflammatoires tels que VCAM-1 contribuent davantage aux lésions vasculaires et à la diminution de la biodisponibilité du monoxyde d'azote. Ces processus physiopathologiques entraînent une hypoxie placentaire et une activation endothéliale systémique, établissant ainsi les manifestations cliniques de la prééclampsie. Plus encore, l'environnement intra-utérin défavorable exerce des effets durables sur la descendance. La réduction du NO dans le sang du cordon, l'expression anormale des molécules d'adhésion et les modifications épigénétiques prédisposent les nouveau-nés à une dysfonction vasculaire précoce et à un risque cardiovasculaire accru au cours de la vie. Ainsi, la prééclampsie représente non seulement une complication maternelle mais aussi un déterminant de la santé cardiovasculaire intergénérationnelle, soulignant la nécessité de stratégies ciblées visant à réduire ses conséquences à long terme chez la descendance.

Mots-clés : Prééclampsie, Stress oxydatif, Dysfonction endothéliale, Modifications épigénétiques, Risque cardiovasculaire.

Abstract

Preeclampsia is a complex hypertensive disorder of pregnancy driven by biochemical and molecular disruptions at the maternal–placental interface. Central mechanisms include oxidative stress, endothelial dysfunction, and angiogenic imbalance, characterized by altered levels of VEGF, PlGF, and sFlt-1. Dysregulation of the renin–angiotensin–aldosterone system, increased production of reactive oxygen species, and pro-inflammatory mediators such as VCAM-1 further contribute to vascular injury and impaired nitric oxide bioavailability. These pathophysiological processes lead to placental hypoxia and systemic endothelial activation, establishing the clinical features of preeclampsia. Importantly, the adverse intrauterine environment has lasting consequences for offspring. Reduced cord blood NO, abnormal expression of adhesion molecules, and epigenetic modifications collectively predispose neonates to early vascular dysfunction and heightened cardiovascular risk later in life. Thus, preeclampsia represents not only a maternal complication but also a determinant of intergenerational cardiovascular health, underscoring the need for targeted strategies to mitigate long-term outcomes in affected offspring.

Keywords : Preeclampsia, Oxidative stress, Endothelial dysfunction, , Epigenetic modifications, Cardiovascular risk.

List of abbreviations

ADMA : Asymmetric Dimethylarginine

AT1-AA : Angiotensin II Type 1 Receptor Agonistic Autoantibodies

BH4 : Tetrahydrobiopterin

CBS : Cystathionine-β-Synthase

CSE / Cth : Cystathionine-γ-Lyase

CVD : Cardiovascular Disease

DBP : Diastolic Blood Pressure

eNOS / NOS : Endothelial Nitric Oxide Synthase / Nitric Oxide Synthase

H_2S : Hydrogen Sulfide

HLA-C / HLA-E / HLA-G : Human Leukocyte Antigen C / E / G

HO / HO-1 / HO-2 : Heme Oxygenase (various isoforms)

Hsp90 : Heat Shock Protein 90

ICAM-1 : Intercellular Adhesion Molecule-1

MMPs / MMP-2 / MMP-9 : Matrix Metalloproteinases (various isoforms)

MPST : 3-Mercaptopyruvate Sulfurtransferase

PAI-1 : Plasminogen Activator Inhibitor-1

PE : Preeclampsia

PI3K : Phosphoinositide 3-Kinase

PlGF : Placental Growth Factor

SBP : Systolic Blood Pressure

sEng : Soluble Endoglin

sFlt-1 : Soluble FMS-Like Tyrosine Kinase-1

TIMP-1 : Tissue Inhibitor of Metalloproteinases-1

VCAM-1 : Vascular Cell Adhesion Molecule-1

VEGF : Vascular Endothelial Growth Factor

WHO : World Health Organization

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Introduction

Introduction

Pregnancy is a distinctive physiological process associated with a wide spectrum of biochemical modifications (Ichipi-Ifukor *et al*., 2013). The success of human pregnancy relies on several fundamental biological processes, including embryo implantation, decidualization, placentation, and parturition, each of which is essential for favorable pregnancy outcomes (Aplin & Ruane, 2017). To meet fetal requirements, the maternal body adapts its physiological and homeostatic mechanisms, leading to elevations in blood glucose, respiratory activity, and cardiac output (Jiang *et al*., 2012). Although most pregnancies culminate in successful deliveries, certain disorders such as preeclampsia—a hypertensive complication of pregnancy—remain major contributors to maternal and perinatal mortality (Arulkumaran & Lightstone, 2013). They are also associated with long-term maternal health sequelae and adverse outcomes related to indicated preterm birth (Abalos *et al*., 2013). Preeclampsia is a highly complex, multisystem syndrome that may involve the renal, neurological, hepatic, and vascular systems (Sperling *et al*., 2015).

Preeclampsia is currently regarded as more than merely a vascular disorder; it encompasses multifactorial pathophysiological mechanisms, including defective early placentation, inadequate remodeling of the spiral arteries, disturbances in immune balance, and oxidative stress resulting from localized hypoxia (Wu *et al*., 2017). Despite decades of research, its precise etiology continues to be unclear, posing persistent challenges for both clinical management and scientific investigation (Balani *et al*., 2025).

offspring health, with a particular focus on cardiovascular disease (CVD). Growing evidence indicates that individuals born to mothers affected by preeclampsia may carry an increased risk of CVD later in life (Balani *et al*., 2025). Although the exact pathways have not yet been fully delineated, biochemical mechanisms are considered to play a central role. Examining these molecular and biochemical foundations is essential for identifying possible preventive and therapeutic targets with implications for maternal and offspring outcomes (Barker *et al*., 1993). This underscores the importance of adopting an integrative approach that combines clinical, physiological, and biochemical perspectives in the study of this disorder.

In this context, the present dissertation aims to provide a narrative review of the biochemical and molecular foundations of preeclampsia, with a particular focus on its long-term impact on offspring cardiovascular health. The work is structured into chapters addressing the theoretical background, molecular mechanisms, cardiovascular consequences for the offspring, and, finally, a critical analysis of current research gaps in this vital field.

Chapter I: Theoretical and Physiological Overview of Preeclampsia

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Preface

Hypertensive disorders during pregnancy (Abalos *et al*., 2013) are defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg (The measurement method is found in Appendix A), and are categorized into four types: preeclampsia, chronic hypertension, chronic hypertension with superimposed preeclampsia, and gestational hypertension (Leanos-Miranda *et al*., 2007). These disorders pose a substantial risk and exert a significant impact on maternal and neonatal health indicators. They are major contributors to maternal and perinatal mortality and lead to long-term maternal health complications, as well as severe outcomes associated with elective preterm births, particularly preeclampsia (PE) (Abalos *et al*., 2013). This type of hypertensive disorder during pregnancy (HDP) is associated with early abnormal placentation, leading to placental insufficiency, oxidative stress, and an imbalance between inflammatory responses and anti-angiogenic processes (Giorgione *et al*., 2024).

I.1. Definition and Classification of Preeclampsia

I.1.1. Clinical Definition

Pre-eclampsia is a progressive pregnancy disorder involving multiple organ systems. Over time, its clinical definition has expanded from a narrow focus on hypertension and proteinuria to a broader framework that acknowledges the disease's complex multi-organ involvement. International guidelines define pre-eclampsia as the new onset of hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or both) accompanied by proteinuria or maternal organ dysfunction after 20 weeks of gestation, or both (Lindsay, 2020).

Organ involvement includes the brain (severe headache, visual disturbances, or eclamptic seizures), liver (epigastric pain or abnormal liver tests), kidneys (abnormal renal function or proteinuria), hematologic system (hemolysis, thrombocytopenia, or coagulopathy), lungs (low oxygen saturation or pulmonary edema), and placenta (fetal growth restriction) (Lindsay, 2020).

I.1.2. Subtypes of Preeclampsia

Based on the gestational age at which clinical signs emerge, pre-eclampsia is classified into two types, with each form displaying distinct pathophysiological mechanisms (Huppertz, 2008):

I.1.2.1. Early-Onset (< 34 weeks)

Early-onset pre-eclampsia is commonly associated with pronounced placental developmental defects, impaired uteroplacental circulation, abnormal Doppler velocimetry of uterine arteries, fetal growth restriction, and poorer maternal and perinatal outcomes (Ness & Sibai, 2006).

I.1.2.2. Late-Onset (≥ 34 weeks)

Conversely, late-onset pre-eclampsia is more frequently linked to metabolic disorders, systemic inflammation, and chronic endothelial dysfunction, often in association with obesity and pre-existing chronic diseases. The uteroplacental compartment in these cases tends to appear normal or only slightly altered. Maternal and neonatal outcomes are usually more favorable due to the proximity to term; however, vigilant monitoring remains essential (Sibai *et al*., 2005).

It can also be divided using 37 weeks as a dividing line into:

I.1.2.3. Preterm preeclampsia (34–37 weeks)

I.1.2.4. term preeclampsia (Beyond 37 weeks) (Saftlas *et al*., 1990)

Preeclampsia is classified according to the severity of hypertensive values during pregnancy into:

I.1.2.5. Severe Preeclampsia

Severe pre-eclampsia is defined by the presence of at least one of the following criteria: systolic blood pressure ≥160 mmHg, diastolic blood pressure ≥110 mmHg, proteinuria of 1 g over 24 hours, or evidence of end-organ involvement **(**Table I.1). Beyond new-onset hypertension and proteinuria, pre-eclampsia may present with a range of nonspecific symptoms such as headache, visual disturbances, vomiting, and epigastric pain. Most women who later experience eclamptic seizures report one or more of these symptoms in the preceding weeks (Knight, 2007).

I.1.2.6. Mild Preeclampsia

In pregnancy, hypertension is considered mild when SBP is at least 140 mmHg or DBP is at least 90 mmHg, with higher thresholds of 150/100 mmHg and 160/110 mmHg defining moderate and severe hypertension, respectively (Leanos-Miranda *et al*., 2007).

Table I.1: Definitions of Preeclampsia and Severe Preeclampsia (Arulkumaran & Lightstone, 2013)

Preeclampsia	Severe Preeclampsia
1. Systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg occurring after 20 weeks of gestation in a previously normotensive woman. AND 2. Proteinuria ≥300 mg in a 24-hour urine collection, or a protein-to-creatinine ratio ≥ 0.3 mg/mg in a random urine specimen, or a dipstick reading of ≥ 2+.	1. Systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg on at least two separate readings spaced 6 hours apart OR 2. Proteinuria ≥1 g in a 24-hour urine collection in the context of preeclampsia OR 3. Evidence of end-organ dysfunction associated with preeclampsia, such as: • Oliguria (<400 mL/day) • Thrombocytopenia • Abnormal liver function tests • Epigastric or right upper quadrant pain (due to hepatic capsule distension) • Neurological or visual disturbances • Pulmonary edema

I.2. Epidemiology and Public Health Significance

Hypertensive disorders complicate up to 15% of pregnancies, while pre-eclampsia (PE) affects approximately 3–10% of expectant mothers (Fox *et al*., 2019; Medjedovic *et al*., 2022). PE contributes substantially to maternal and neonatal morbidity and mortality, particularly in resource-limited settings. It complicates approximately 1.48% of births globally and accounts for nearly 500,000 neonatal and 76,000 maternal deaths annually, and 1.5–2 million neonatal deaths annually (Brown *et al*., 2018; Von Dadelszen & Magee, 2016) with early-onset cases associated with worse outcomes (Oluwole *et al*., 2022). Marked disparities exist, with women in low-resource countries at a greater risk than those in high-resource nations (Poon *et al*., 2019).

I.3. Physiological Adaptations in Normal Pregnancy: Vascular, Placental, and Immune Aspects

I.3.1. Placental Development

I.3.1.1. Definition

The placenta, a fetal organ, begins forming shortly after fertilization and undergoes continual adaptation during gestation to meet the metabolic requirements of the fetus (Turco & Moffett, 2019). Serving as the principal communication interface between mother and fetus, it mediates the transfer of signals, nutrients, wastes, gases, and external factors (Rosenfeld, 2015). They also play a pivotal role in ensuring the health of both the fetus and the mother (Brosens *et al*., 2011), This protects the fetus from maternal immune responses by establishing immunological tolerance; It also maintains pregnancy by synthesizing and releasing hormones (Kojima *et al*., 2022). Consequently, the placenta plays a decisive role in developmental outcomes, as embryogenesis cannot proceed without a fully functional placenta (Brosens *et al*., 2011).

I.3.1.2. Structure of the Placenta

The mature placenta, is a discoid organ averaging 22 cm in diameter, 2.5 cm in thickness at the center, and weighing approximately 500 grams. Its fetal-facing side, the chorionic plate, anchors the umbilical cord, while the basal plate interfaces with the maternal endometrium. Between these two lies the intervillous space, occupied by 30–40 highly branched fetal villous trees. Each originates from a stem villus anchored to the chorionic plate, branching out to form spherical lobules 1–3 cm in diameter. These lobules are centered around the outlets of maternal spiral arteries, through which maternal blood flows, percolates among the villi, and exits via uterine veins. Each lobule thus functions as a discrete maternal–fetal exchange unit.

The terminal branches of the villous trees, or terminal villi, offer a large exchange surface (12–14 m^2 at term) and are densely vascularized by fetal capillaries. Local capillary dilations, called sinusoids, reduce the diffusion distance between maternal and fetal blood to about 2–3 μm by bringing fetal endothelium close to the overlying trophoblast. These vasculosyncytial membranes structurally resemble alveoli and are considered key sites of nutrient and gas exchange (Simpson *et al*., 1992).

I.3.1.3. Early placental development

Human placental development relies on tightly regulated interactions between the placental trophoblast lineages (TSCs) and the maternal endometrial tissue. The process begins when the

polar trophectoderm of the blastocyst (the trophectoderm (TE), which forms the trophoblast epithelium, and the inner cell mass (ICM) (Marikawa & Alarcón, 2009)) adheres to the receptive endometrial epithelium around days 5–6 post-fertilization (dpf). Once stable contact is established, initial blastocyst–uterine interactions trigger gene expression in the trophectoderm (Aplin & Ruane, 2017) and lead to the development of primary invasive syncytial structures (Aplin et al., 2018). Around days 6–8, the syncytiotrophoblast penetrates the endometrial layer (Figure I.1), triggering transformation of the endometrium into decidua (Schlafke & Enders, 1975). Cells of the blastocyst wall that remain unfused differentiate into cytotrophoblasts, which expand the chorionic syncytiotrophoblast. By the end of implantation, the syncytium envelops the entire gestational sac. Fluid-filled lacunae emerge, merging into a network of trabeculae that invade uterine glands to absorb their secretions (Hertig *et al*., 1956). At the same time, the embryo undergoes differentiation into a bilaminar disc composed of epiblast and hypoblast layers, and both the amniotic cavity and the primary yolk sac begin to form (Cindrova-Davies & Sferruzzi-Perri, 2022). From ~12 dpf, proliferating cytotrophoblasts invade the syncytium, initiating primary villi formation. Simultaneously, a new embryonic-derived layer—the extraembryonic mesoderm—begins to emerge (Cindrova-Davies & Sferruzzi-Perri, 2022) (Figure I.2). Some cells form anchoring columns that attach the placenta to the decidua and expand laterally to form the cytotrophoblast shell—precursor to the basal plate (Shahbazi *et al*., 2016). By ~17–18 dpf, extraembryonic mesenchyme enters the villi to form secondary villi. Hemangioblastic clusters soon appear, giving rise to fetal vasculature and tertiary villi (John D. Aplin et al., 2015), rapidly expanding the villous tree (Cindrova-Davies & Sferruzzi-Perri, 2022). At the maternal-fetal boundary, some cytotrophoblasts differentiate into non-proliferative extravillous trophoblasts (EVTs) (Vento-Tormo *et al*., 2018), This is by day 18, when placental villi have formed (Aplin *et al*., 2018). and a significant EVT population has already begun invading the maternal stroma (Aplin & Jones, 2008) (Figure I.3).

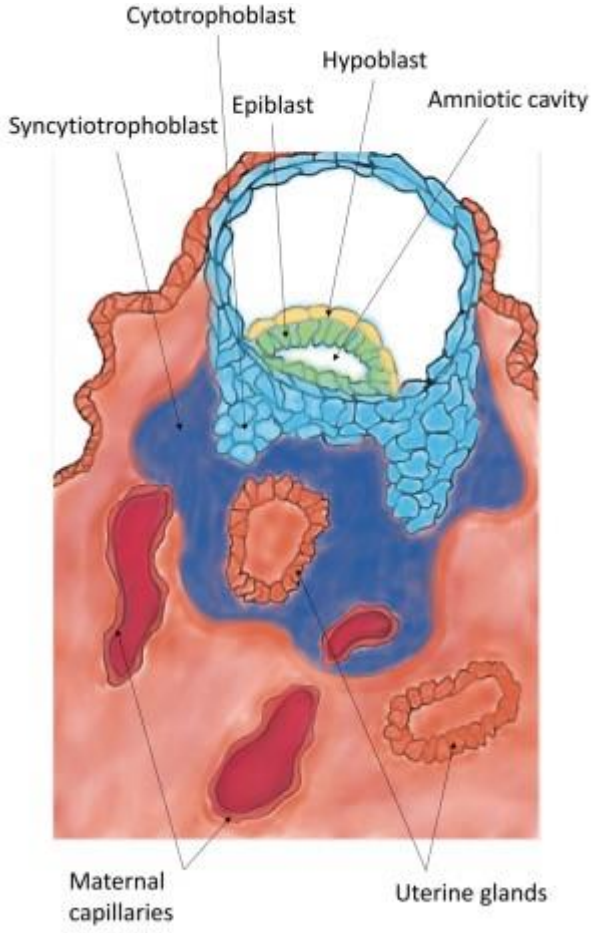

Figure I.1: Early stages of placental development (~6–8 days post-fertilization) (Cindrova-Davies & Sferruzzi-Perri, 2022).

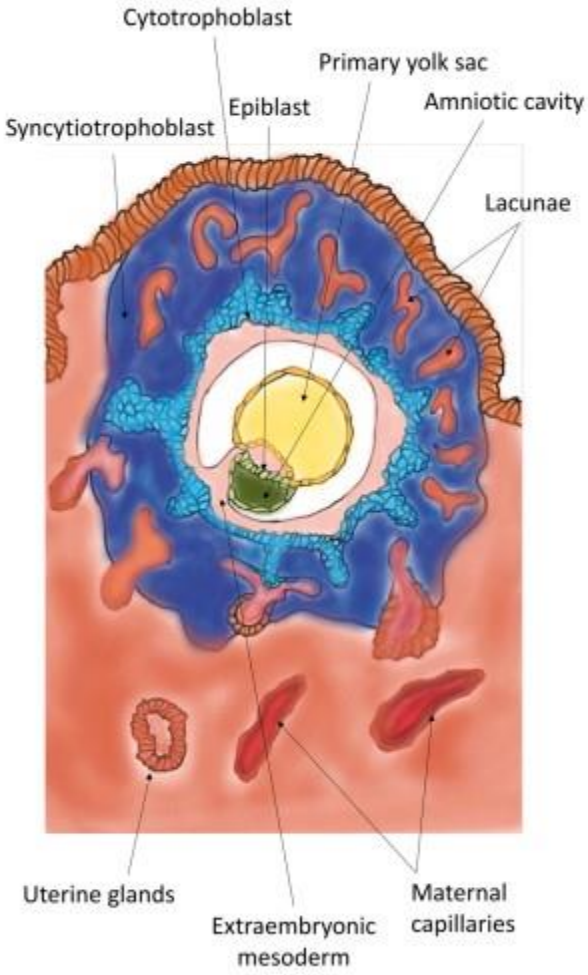

Figure I.2: Early placental development – primary villus formation (~12–14 days post-fertilization) (Cindrova-Davies & Sferruzzi-Perri, 2022).

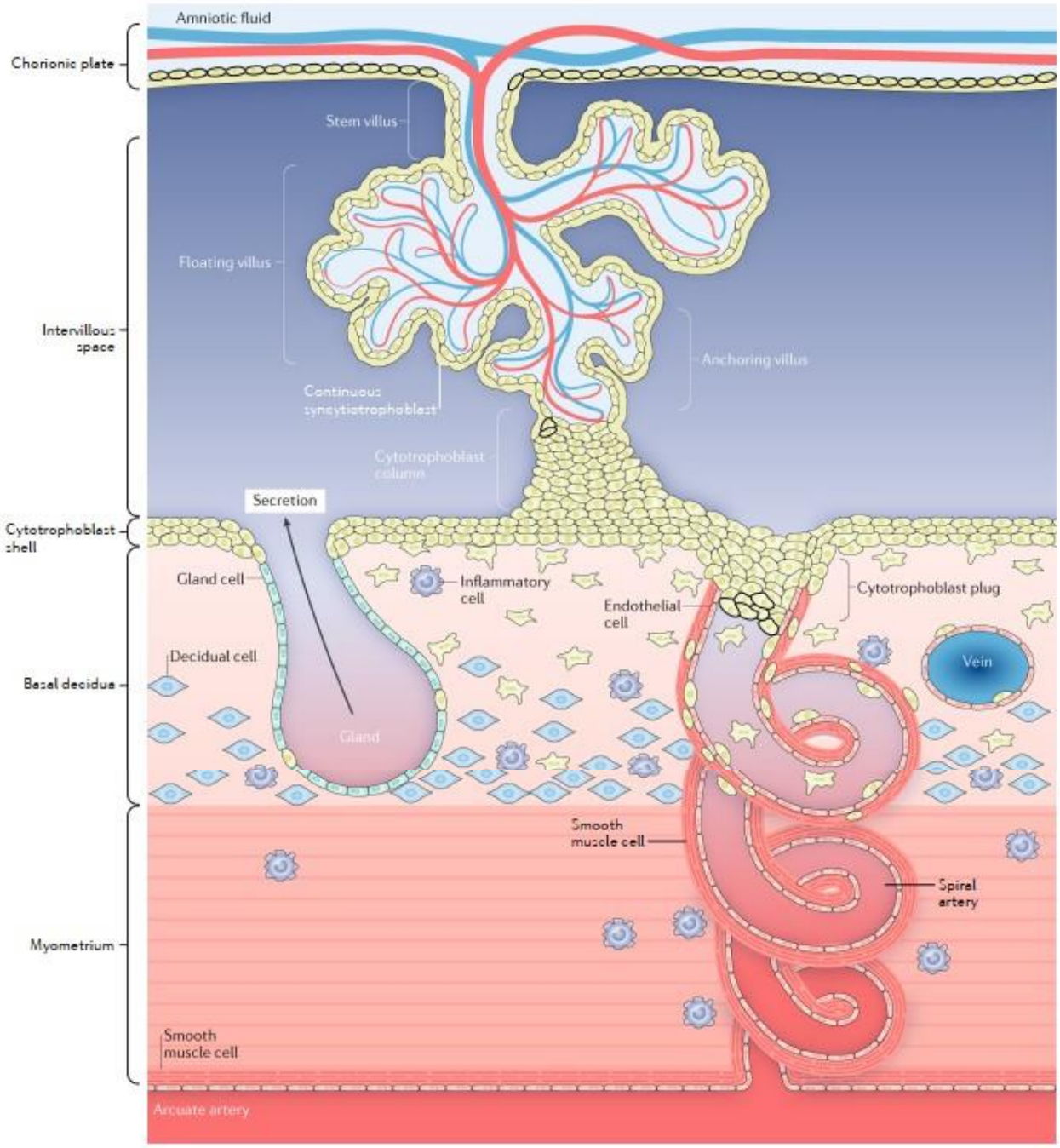

Figure I.3: Structure of the placenta and placental bed during the first trimester (Aplin *et al*., 2020).

I.3.2. Vascular Adaptations

I.3.2.1. Maternal Blood Supply Formation

During the first 11 weeks of pregnancy, while histiotrophic support predominates, the remodeling of decidual spiral arteries near and beyond the trophoblast plugs begins. uNK cells are

recruited into the smooth muscle layers of the decidua basalis in response to cytokines released by endothelial cells, which are themselves stimulated by EVT-derived signals like IL-6 and IL-8 (Velicky *et al*., 2018). These uNK cells initiate breakdown of the vascular extracellular matrix, a process furthered by invasive trophoblasts (Choudhury *et al*., 2019). Smooth muscle cells are lost via dedifferentiation and apoptosis(Harris *et al*., 2006), and the vessel wall is replaced by fibrinoid material embedding residual trophoblasts .This remodeling renders arteries non-contractile and dilated (Harris *et al*., 2010). The upstream softening allows gradual displacement of trophoblast plugs, facilitating blood flow into the intervillous space, though still limited by vasoconstriction in radial arteries (Roberts et al., 2017). After this, the placental barrier becomes haemochorial. (Hempstock *et al*., 2003).

I.3.2.2. Formation of the Placental–Fetal Circulatory System

From the earliest stages of villus formation, the placenta establishes multiple adhesion points to the uterine (Enders *et al*., 2001) and Proper villous maturation according to gestational age is fundamental for ensuring placental function that meets the oxygen and nutrient requirements of the fetus (Turowski & Vogel, 2018). Initial villous development begins around day 8 post-fertilization when protrusions of primitive syncytiotrophoblast extend into the decidua. Underneath, cytotrophoblast proliferation gives rise to the first villous structures By day 12, extraembryonic mesenchyme infiltrates the villi, forming mesenchymal cores. Around day 15, endothelial cell cords become evident within these cores (Jones *et al*., 2015), often located just beneath the trophoblast basement membrane. The first fetal capillaries appear by days 18–20, along with Hofbauer macrophages, which support early vasculogenesis (Jones *et al*., 2015).

The development of tertiary villi signifies the completion of the embryonic phase of placental formation. In the ensuing weeks up to delivery, villous maturation progresses through elongation, branching, and transformation of the stromal core, fetal vasculature, and the trophoblastic (chorionic epithelial) layer. This maturational process includes (Benirschke *et al*., 2012):

Beginning in the third week of development, founder endothelial cells and vascular cords emerge, leading to rapid expansion of a vascular network within the villous mesenchyme and the chorionic plate (John D Aplin *et al*., 2015). By day 22, endothelial cords interconnect and elongate parallel to the villus axis, establishing primitive vascular branches (Demir *et al*., 2004). These are enveloped by perivascular and smooth muscle cells to form contractile vessels Around day 32, placental vessels link to the embryonic circulation via the umbilical cord, which develops from the allantoic duct (Hafez, 2017).

To simplify this initially complex network, vascular pruning must occur during the first trimester. Further remodeling includes complete regression of blood vessels in areas adjacent to the decidua capsularis (the implantation roof) (see Appendix B)., resulting in formation of an avascular, smooth chorion by the end of the first trimester (John D Aplin *et al*., 2015).

By week 12, vessel muscularization begins in the chorionic plate and extends into stem villi. Sites of red blood cell production appear in both the chorionic plate and the villi in early pregnancy (John D Aplin *et al*., 2015). These nucleated red blood cells likely enter embryonic circulation once the heart starts beating around day 22 post-conception, although placental blood flow is not typically detectable via ultrasound until about week 5 (Burton & Jauniaux, 2018). As gestation proceeds, vascular morphogenesis continues, giving rise to intermediate villi that bridge stem villi from the chorionic plate to terminal villi—key zones of nutrient and gas exchange (Burton *et al*., 2009) (see Appendix C).

In the third trimester, capillaries within terminal villi form loops that approach very close to the trophoblast basement membrane (Burton & Jauniaux, 2018). As the villous tree grows and branches, it becomes increasingly populated by vessels extending from the chorionic plate and presumptive stem villi (Aplin *et al*., 2020). The vascular network expands via branching angiogenesis in the second trimester and non-branching angiogenesis in the third, forming terminal capillary loops that optimize maternal-fetal exchange. Meanwhile, the cytotrophoblast layer thins and flattens against the syncytiotrophoblast, facilitating closer capillary contact with the exchange surface (Boss *et al*., 2018) (Figure I.6).

As these events progress, mature villous architecture is established: fetal blood vessels and macrophages reside in a loose mesenchymal core encased by a bilayer of specialized trophoblasts. The inner cytotrophoblast layer proliferates rapidly and either fuses into the syncytiotrophoblast—responsible for maternal-fetal exchange—or differentiates into extravillous trophoblasts that remodel maternal spiral arteries for adequate perfusion (Boss *et al*., 2018).

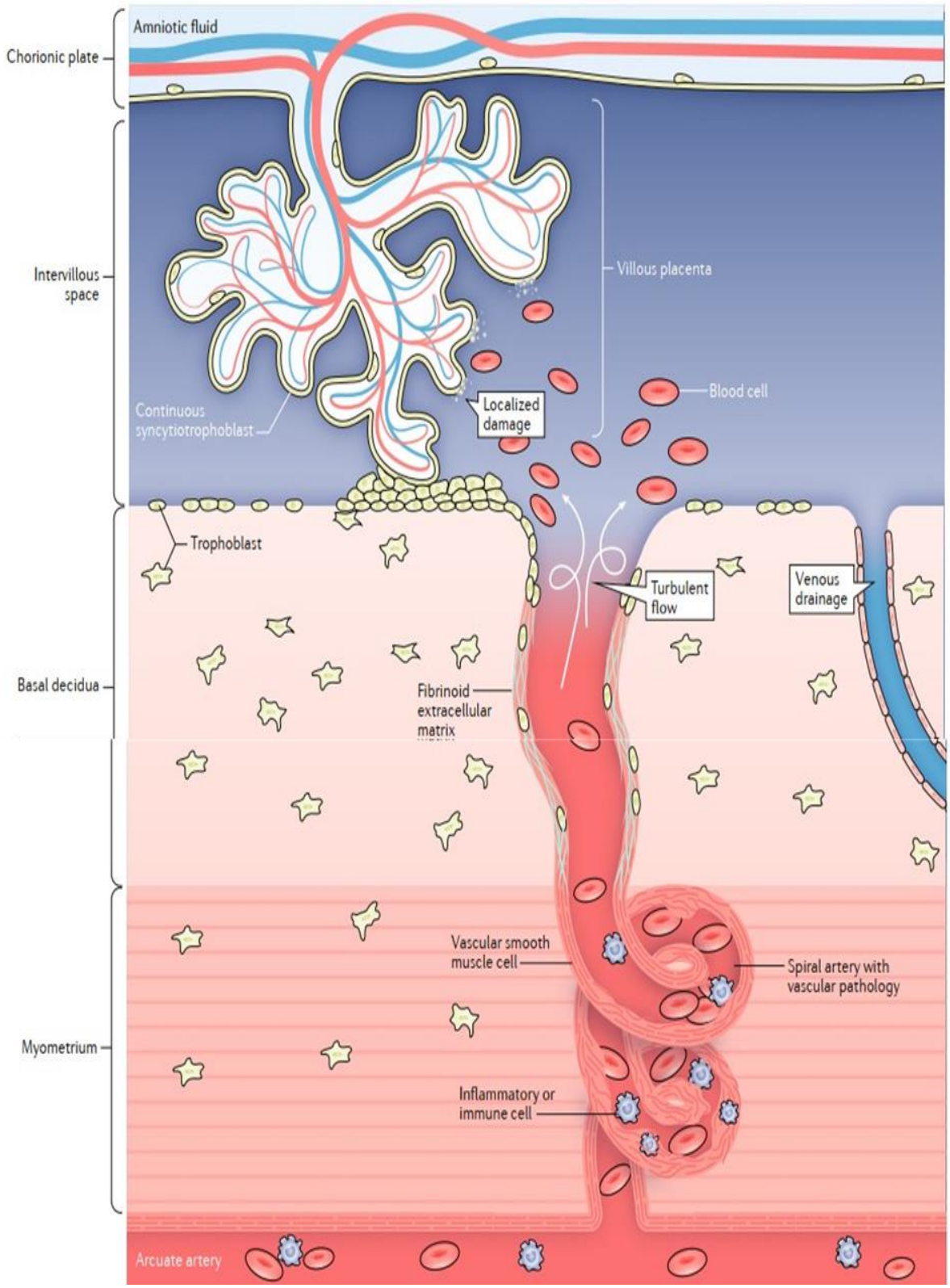

Figure I.4: Spiral Arterial Pathology in the Near-Term Placenta and Placental Bed (Aplin et al., 2020).

Chapter II: Biochemical and Molecular Mechanisms Underlying Preeclampsia

Chapter II. Biochemical and Molecular Mechanisms Underlying Preeclampsia

Preface (The Two-Stage Model of Preeclampsia)

Current understanding of preeclampsia recognizes distinct differences between its early- and late-onset forms. Given the still-limited insight into this disorder, the modified "two-stage model" has emerged as one of the most widely accepted frameworks to explain its pathogenesis (Chiang *et al*., 2024). Although the exact cellular and molecular mechanisms remain incompletely understood, the condition is believed to evolve through two successive stages (Chang *et al*., 2023).

In this model, the first phase—termed the "placental stage"—is defined by defective spiral artery remodeling, resulting in reduced placental blood flow and ischemia (Pankiewicz *et al*., 2021). The second phase represents the phase where impaired uteroplacental blood flow interacts with maternal predispositions, resulting in widespread pathophysiological disturbances across multiple organ systems, reflecting inadequate perfusion. These maternal manifestations are primarily driven by systemic endothelial dysfunction and injury (Chang *et al*., 2023).

The transition from Stage 1 to Stage 2 has been linked to several mechanisms, including microparticles generated by syncytiotrophoblast apoptosis, inflammatory mediators, activation of the renin–angiotensin system, angiogenic imbalance involving VEGF, PlGF, and the anti-angiogenic factor sFlt-1, as well as oxidative stress accumulation (Kornacki *et al*., 2023).

II.1. Maternal Predisposing Factors

II.1.1. Predisposing Genetic

Multiple gene mutations have been linked to preeclampsia (PE) (Figure II.1), and increased decidual mRNA expression of candidate maternal susceptibility genes correlates with disease severity (Yong *et al*., 2014). Early studies reported downregulation of 31 out of 36 placental genes in PE (Founds *et al*., 2009). A more recent review identified 250 differentially expressed genes across three datasets, including 228 upregulated and 22 downregulated genes in PE placenta compared with normal placenta. Candidate genes highlighted include LEP, NRIP1, SASH1, and ZADHHC8P1. Severe PE is also associated with elevated decidual mRNA levels of ACVR1, INHBB, ERAP1, ERAP2, LNPEP, COL4A1, and COL4A2; increased expression of INHA, INHBB, COL4A1, and COL4A2 correlates with earlier onset and delivery (Yong *et al*., 2014).

II.1.2. Demographic, dietary, and environmental factors

Demographic and lifestyle factors, including maternal age, ethnicity, BMI, preexisting medical conditions, PE history, primiparity, and multiple pregnancy, affect PE risk. Both very young (<16 years) and older (>40 years) mothers are more vulnerable (Kanagal *et al*., 2014). Maternal body weight is another key determinant: prevalence rises from 3% in women with normal BMI (18.5–24.9) to 7% in overweight women (BMI 30–34.9) and 13% in those with extreme obesity (BMI ≈50) (Spradley *et al*., 2015). Nutritional factors such as high caloric and salt intake or inadequate magnesium and calcium consumption have also been implicated (Schoenaker *et al*., 2014). Low serum levels of magnesium, calcium, vitamin D, and zinc are linked to PE (Xu *et al*., 2009). Environmental exposures, including fine particulate matter and nitrogen dioxide, contribute additional risk (Pedersen *et al*., 2014). Pre-existing medical and psychological conditions—cardiovascular disease, chronic respiratory illness, diabetes, renal disease, systemic lupus erythematosus, prior reproductive surgery, antepartum hemorrhage, and mental stress—further heighten susceptibility (Qu & Khalil, 2020) (Figure II.1) **(**Table I.2).

Table II.1:Risk Factors for Pre-eclampsia and Associated Unadjusted Relative Risks. This table presents various risk factors for pre-eclampsia in descending order of pooled unadjusted relative risk (RR), as reported by two systematic reviews (Bartsch *et al*., 2016; Duckitt & Harrington, 2005) (The interpretation of relative risks (RRs) may vary depending on clinical experience (Committee, 2011)).

Risk Factor	Pooled Unadjusted RR (95% CI) (Systematic Review 1)	Unadjusted RR (95% CI) (Systematic Review 2)
History of pre-eclampsia	8.4 (7.1–9.9)	7.19 (5.85–8.83)
Chronic hypertension	5.1 (4.0–6.5)	–
Pre-gestational diabetes	3.7 (3.1–4.3)	3.56 (2.54–4.99)
Maternal age <17 years	–	2.98 (0.39–22.76)
Multiple pregnancy	2.9 (2.6–3.1)	2.93 (2.04–4.21) if twin; 2.83 (1.25–6.40) if triplet
Family history of pre-eclampsia	–	2.90 (1.70–4.93)

Antiphospholipid syndrome	2.8 (1.8–4.3)	9.72 (4.34–21.75)
BMI >30 kg/m² (obesity)	2.8 (2.6–3.1)	–
Systemic lupus erythematosus	2.5 (1.0–6.3)	–
History of stillbirth	2.4 (1.7–3.4)	–
Nulliparity (first pregnancy)	2·1 (11·9–2·4)	2.91 (1.28–6.61)
Previous placental abruption	2.0 (1.4–2.7)	–
Use of assisted reproductive technologies	1.8 (1.6–2.1)	–
Chronic kidney disease	1.8 (1.5–2.1)	–
Maternal age >40 years	1.5 (1.2–2.0)	1.68 (1.23–2.29) if primiparous; 1.96 (1.34–2.87) if multiparous
Fetal growth restriction	1.4 (0.6–3.0)	–
Maternal age >35 years	1.2 (1.1–1.3)	–

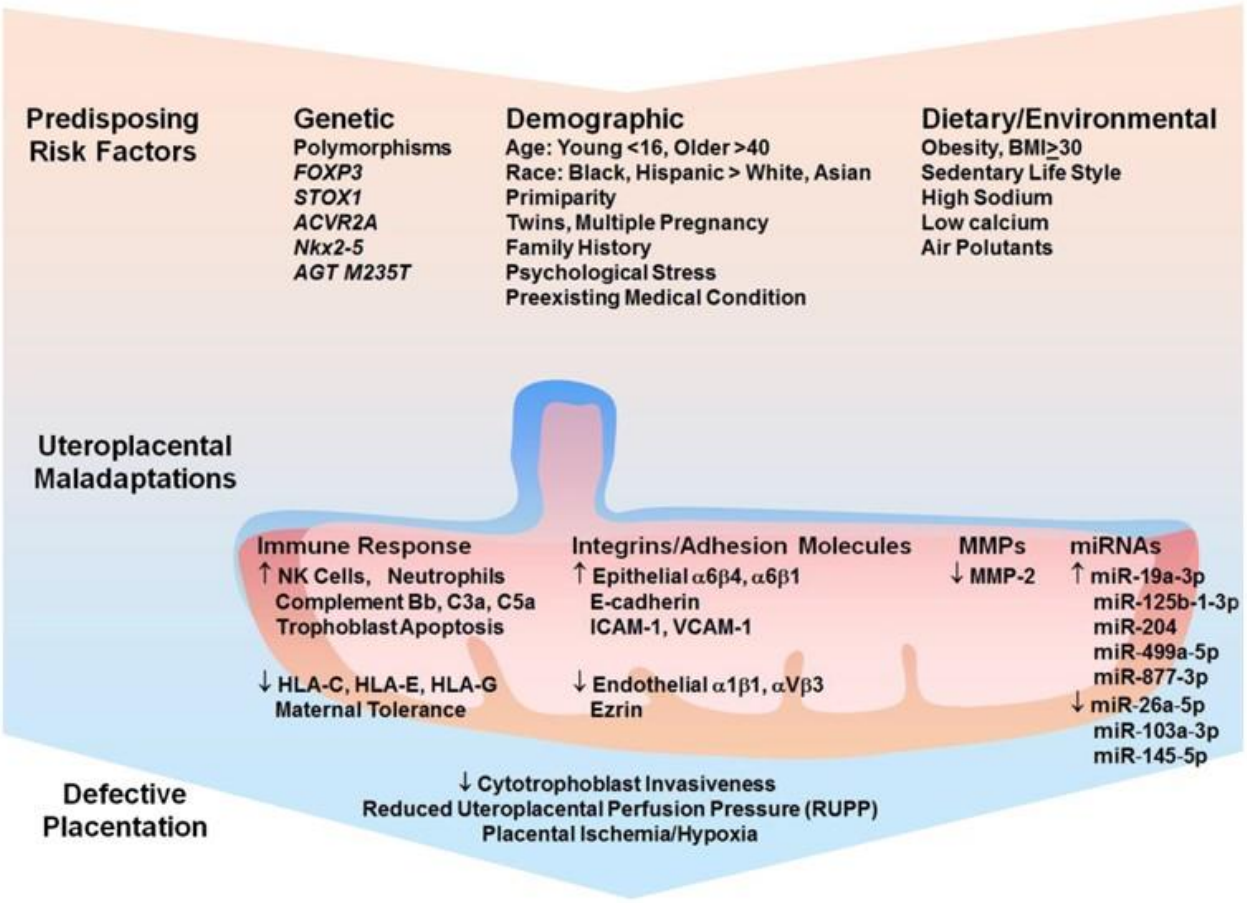

Figure II.1: Risk factors, uteroplacental maladaptations, and placental ischemia. Predisposing genetic polymorphisms, demographic determinants, obesity, physical inactivity, a high-sodium/low-calcium diet, and environmental pollutants may disrupt the uteroplacental immune response, integrins and adhesion molecules, matrix metalloproteinases (MMPs), and microRNAs. These maladaptations contribute to abnormal placentation and the development of placental ischemia and hypoxia. BMI: body mass index; HLA-C, -E, -G: histocompatibility complex molecules; NK: natural killer cells (Qu & Khalil, 2020).

II.2. Placental Dysfunction (Uteroplacental Maladaptations and Defective Placentation in Preeclampsia)

II.2.1. Uteroplacental Integrins and Impaired Trophoblast Invasion in Preeclampsia

Trophoblast invasion and the extensive remodeling of spiral arteries are partly controlled by integrins and other adhesion molecules (Figure II.2). At early stages, cytotrophoblasts express epithelial adhesion proteins such as integrins α6β4, α6β1, and E-cadherin. During normal pregnancy, a transition known as vascular mimicry or pseudovasculogenesis occurs, allowing cytotrophoblasts to adopt an endothelial-like phenotype, replacing epithelial markers with endothelial integrins such as α1β1 and αVβ3 (McMaster *et al*., 2004). It has also been

demonstrated that the proper expression of α1β1 integrin regulates trophoblast fusion within endothelial networks through signaling pathways involving galectin-1, tissue inhibitor of metalloproteinases-1 (TIMP-1), plasminogen activator inhibitor-1 (PAI-1), and the production of MMP-2 and MMP-9 (Xu *et al*., 2020). These integrin shifts and associated invasive mechanisms appear to be disrupted in preeclampsia. In early placentas, hypoxia enhances integrin α5 and fibronectin expression while suppressing α1 expression (Iwaki *et al*., 2004).

In normal gestation, endothelial adhesion molecules ICAM-1 and VCAM-1 are downregulated, reducing leukocyte adhesion and maintaining spiral artery blood flow. However, in preeclampsia, plasma levels of soluble ICAM-1 and VCAM-1 are elevated, reflecting increased endothelial expression that enhances leukocyte attachment and further restricts blood flow (Fei *et al*., 2012) (Figure II.1).

II.2.2. Uteroplacental MMPs and Impaired Placental Vascularization in Preeclampsia

Matrix metalloproteinases (MMPs) are zinc-dependent proteases that degrade extracellular matrix proteins and contribute to endometrial remodeling during the menstrual and estrous cycles, as well as uterine remodeling in pregnancy (Ulbrich *et al*., 2011). Among them, MMP-2 and MMP-9 (Montagnana *et al*., 2009). MMP-2 and MMP-9 is abundantly expressed in invading extravillous trophoblasts (Isaka *et al*., 2003; Qiu *et al*., 2004).

Evidence further supports their role in trophoblast invasion of the decidua: inhibition of MMP-9 reduces first-trimester trophoblast invasiveness (Yu *et al*., 2015). Genetic studies have reported polymorphisms in MMP-2 and MMP-9, along with reduced placental MMP-9 expression in PE (Omran *et al*., 2011) (Figure II.2).

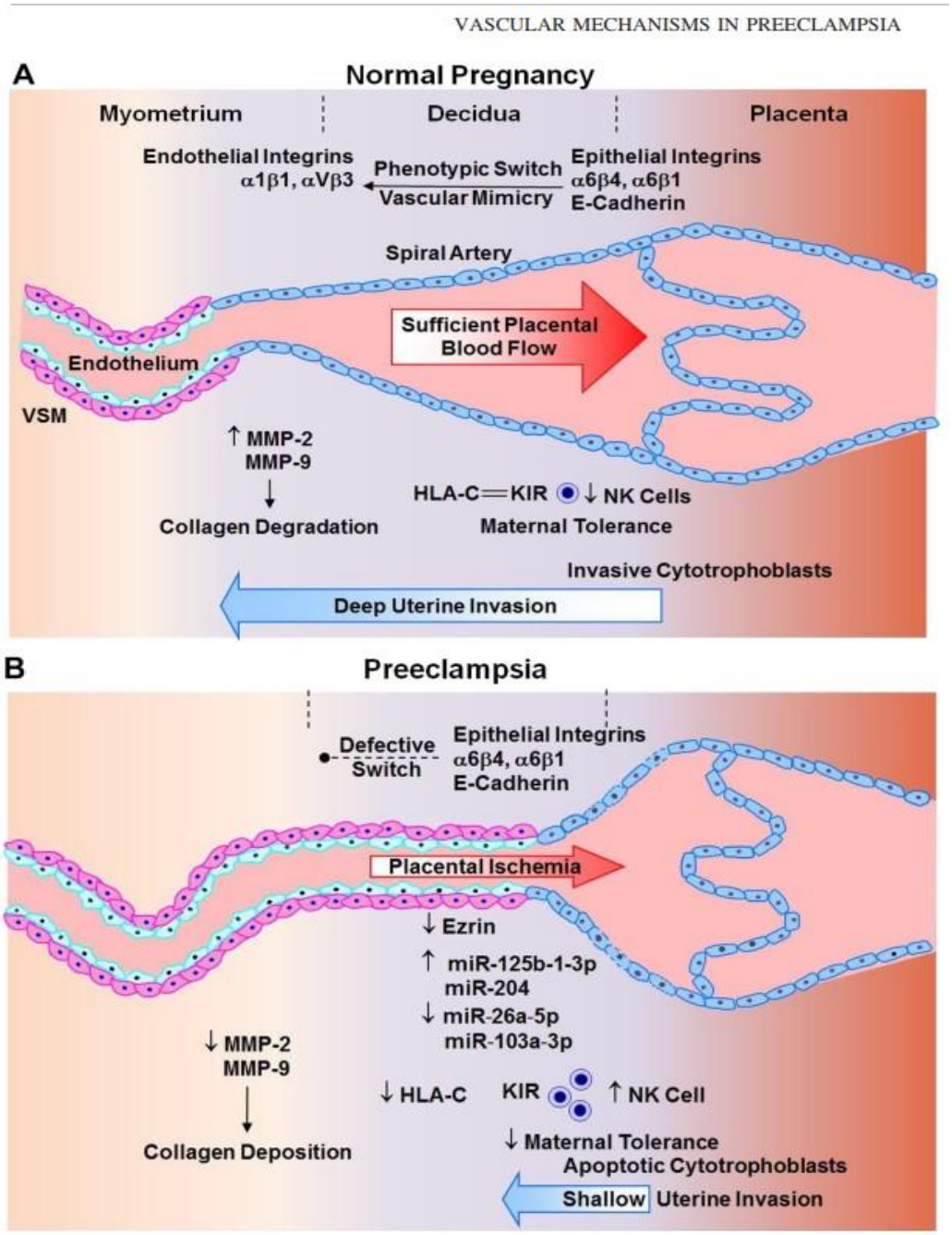

Figure II.2: Defective placentation in preeclampsia. A**:** In normal pregnancy, the phenotypic switch from epithelial to endothelial integrins, coupled with enhanced matrix metalloproteinase (MMP) expression and activity, and the interaction of HLA-C with its killer-cell immunoglobulin-like receptor (KIR), results in reduced natural killer (NK) cell activity. These mechanisms collectively promote deep trophoblast invasion into spiral arteries within the decidua, collagen breakdown, uteroplacental remodeling, and increased maternal immune tolerance, ultimately ensuring adequate placental vascularization and blood supply. **B:** In preeclampsia, failure of the integrin phenotypic switch, reduced MMP levels, and heightened NK cell activity lead to cytotrophoblast apoptosis, shallow invasion of spiral arteries restricted to the superficial decidua, excessive collagen deposition, growth-limiting vascular remodeling, and maternal immune intolerance. These abnormalities culminate in defective placentation and placental ischemia. VSM: vascular smooth muscle (Qu & Khalil, 2020).

II.3. Angiogenic and Anti-Angiogenic Imbalance

II.3.1. Pro-angiogenic factors (Placental Growth Factor (PlGF) and Vascular Endothelial Growth Factor (VEGF))

Placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) are pro-angiogenic molecules secreted by trophoblasts. PlGF, a glycosylated dimeric protein, is essential for placental vascular development during early gestation, supporting trophoblast proliferation, differentiation, and invasion into maternal decidua (Creswell *et al*., 2023). Its maternal plasma levels are low in the first trimester, begin rising around week 11–12, peak at week 30, and decline thereafter (Chau *et al*., 2017). Although primarily produced by the placenta, PlGF is also expressed at lower levels in tissues including the heart, skeletal muscle, lungs, liver, bone, and thyroid (Chau *et al*., 2017).

VEGF is critical for maintaining endothelial cell integrity, especially in fenestrated endothelium of organs such as the brain, liver, and glomeruli—key sites affected in preeclampsia. Both PlGF and VEGF are antagonized by the soluble receptor sFlt-1, which binds these factors and prevents their interaction with cell-surface receptors, thereby lowering the circulating free forms (Rana *et al*., 2019) (Figure II.3).

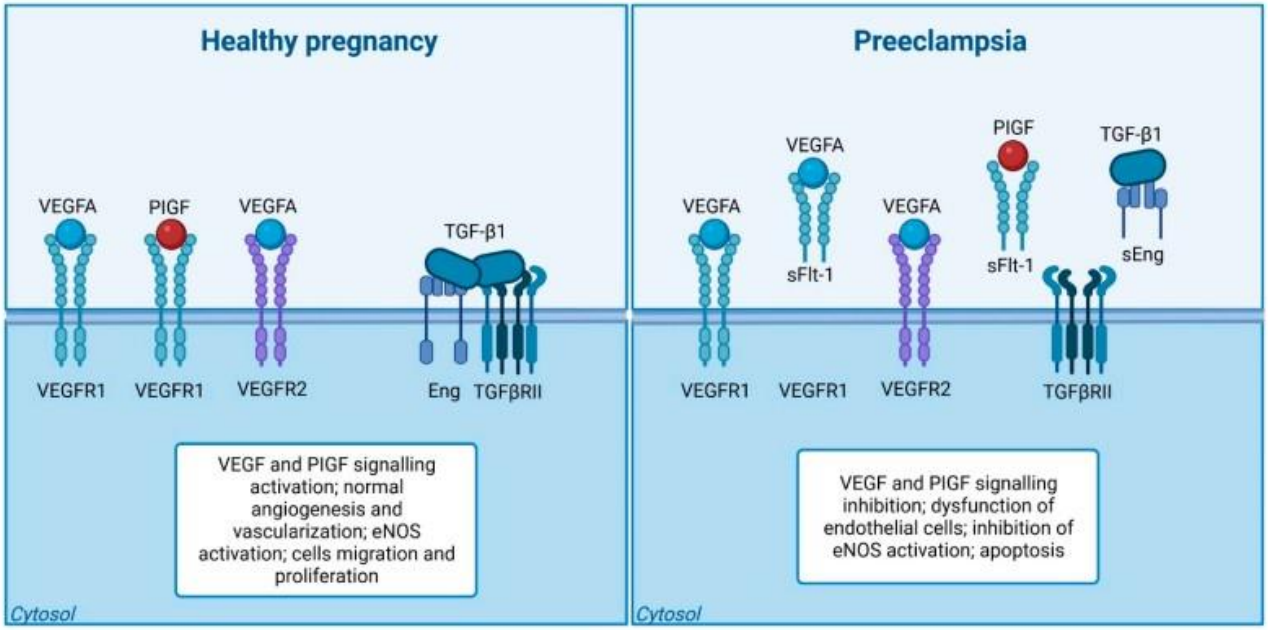

Figure II.3: The Disrupted Balance: Angiogenic Imbalance in Preeclampsia. In normal pregnancy, vascular stability and angiogenic balance are maintained by adequate signaling of vascular endothelial growth factor (VEGF) and transforming growth factor-β1 (TGF-β1). In preeclampsia, however, the

placenta releases excessive amounts of the antiangiogenic proteins sFlt-1 and sEng, which block VEGF and TGF-β1 signaling in the maternal vasculature. This interference disrupts endothelial function, leading to reduced nitric oxide production and vascular dysfunction. Figure created with BioRender.com (Rybak-Krzyszkowska *et al*., 2023).

II.3.2. Anti-angiogenic factors (sFlt-1, sEng)

II.3.2.1. Soluble FMS-Like Tyrosine Kinase-1 (sFlt-1)

Flt-1 is a soluble anti-angiogenic protein that exerts its inhibitory function by binding to and neutralizing the pro-angiogenic molecules PlGF and VEGF, thereby contributing to endothelial dysfunction [3]. Structurally, sFlt-1 represents a splice variant of the membrane-bound receptor Flt-1 (VEGFR-1) and has a molecular weight of approximately 100 kDa, lacking both the transmembrane and intracellular domains of the full receptor (Armaly *et al*., 2018) (Figure II.3).

The placenta is the principal source of circulating sFlt-1 in maternal blood, and elevated production of this protein is a critical driver in the pathogenesis of preeclampsia (PE). Increased levels of sFlt-1 block the biological activities of VEGF and PlGF, promoting disease development (Rybak-Krzyszkowska *et al*., 2023).

II.3.2.2. Soluble Endoglin (sEng)

Soluble endoglin (sEng) is a 65 kDa placental-derived isoform of the transmembrane glycoprotein endoglin. It functions as an antiangiogenic factor by serving as a co-receptor for TGF-β1 and TGF-β3, with high expression in endothelial cells and trophoblasts. While TGF-β normally promotes anti-inflammatory responses and eNOS expression, excess sEng acts as an endogenous inhibitor of TGF-β1, disrupting the signaling pathway, impairing eNOS activation, and limiting vasodilation. This disturbance compromises vascular homeostasis in a manner analogous to the antagonistic effects of sFlt-1 on VEGF, leading to vascular dysfunction and hypertension (Margioula-Siarkou *et al*., 2021) (Figure II.3).

II.3.3. Endothelial and Vascular Dysfunction

II.3.3.1. Nitric Oxide Pathway (The nitric oxide (NO)/nitric oxide synthase (NOS) system)

Nitric oxide (NO), generated by endothelial cells, is a gaseous signaling molecule with potent vasodilatory properties. It plays a key role in vascular homeostasis by regulating angiogenesis, neovascularization, vascular tone, and systemic blood pressure. NO serves as a critical mediator of angiogenic signaling, modulating the activity of VEGF, PlGF, and TGF-β to promote

endothelial proliferation and migration. Expression of eNOS is upregulated by these angiogenic factors (Ahmed, 2011).

In endothelial cells, VEGF binds to its receptor (VEGFR), leading to the activation of a specific isoform of eNOS, regulated by Ca^{2+}/calmodulin. This activation occurs via three mechanisms (Figure II.4):

1. phosphorylation of NOS through the PI3K–Akt pathway.
2. Induction of calcium flux.
3. Recruitment of heat shock protein 90 (Hsp90).

Once activated, eNOS catalyzes the conversion of L-arginine to L-citrulline and NO, which in turn promotes angiogenesis and vasculogenesis.

In preeclampsia, however, this VEGF-dependent signaling is impaired due to elevated circulating levels of sFlt-1 and sEng alongside reduced PlGF expression (Armaly *et al*., 2018). Excessive sFlt-1 and sEng antagonize VEGF-, TGF-β-, and PlGF-induced NO production, reducing vasodilation and contributing to hypertension in PE patients. Reduced NO bioavailability is a key driver of endothelial dysfunction, and inhibition of eNOS by sFlt-1 and sEng provides a molecular explanation for elevated mean arterial pressure. Thus, suppression of VEGF-mediated eNOS activation may impair angiogenesis and exacerbate hypertension (Venkatesha *et al*., 2006).

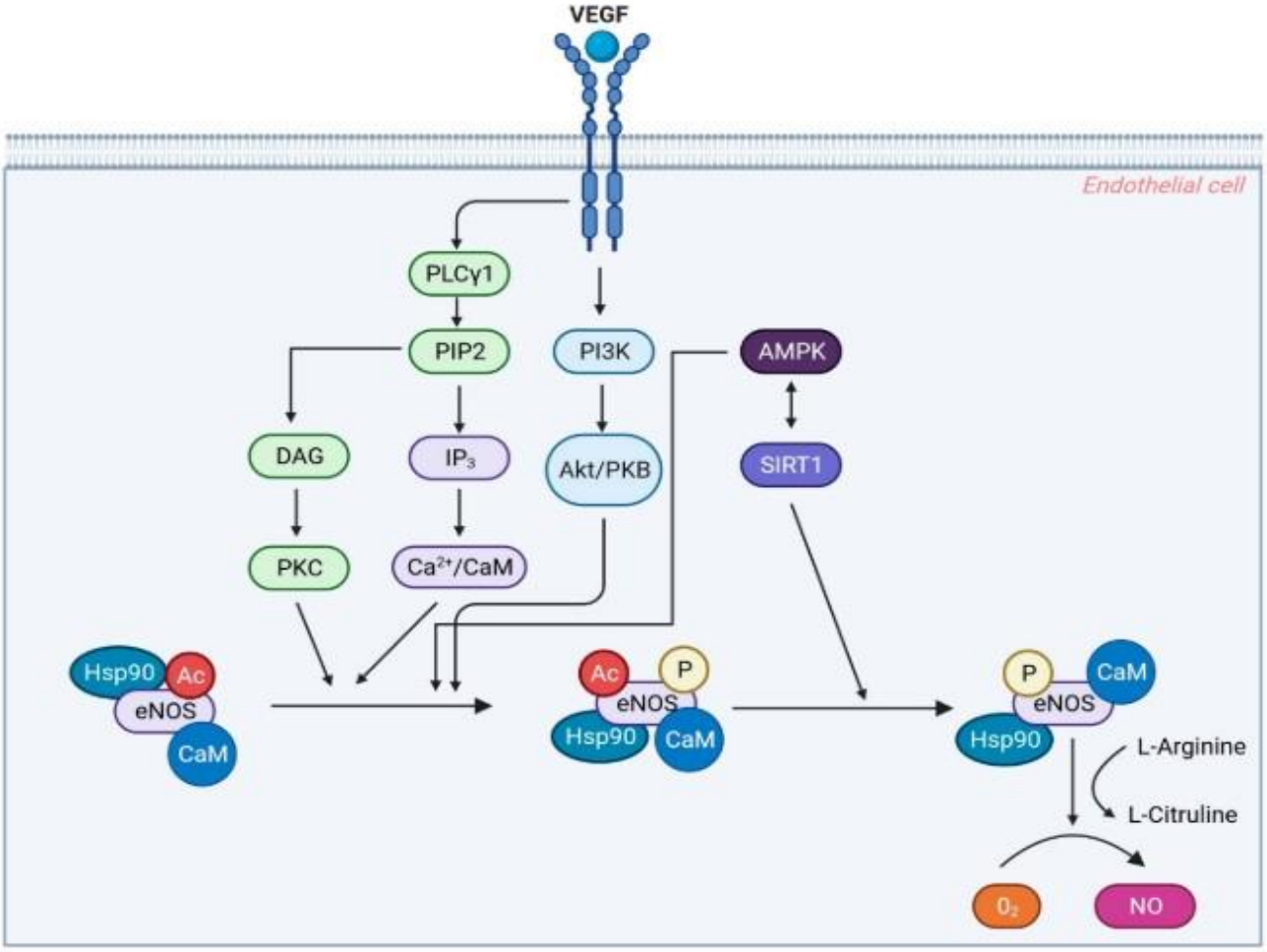

Figure II.4: Regulation of Endothelial Nitric Oxide Synthase (eNOS) through VEGF Signaling. When VEGF binds to VEGFR2, receptor dimerization and activation of tyrosine kinase domains occur, initiating autophosphorylation and downstream signaling. These pathways converge to regulate nitric oxide (NO) synthesis.Activation of VEGFR2 triggers the PI3K/Akt pathway, elevating intracellular Ca2+ levels, which facilitate the binding of calmodulin (CaM) to eNOS, thereby promoting its activation. Concurrently, VEGFR2 signaling activates PLCγ, leading to the hydrolysis of PIP2 into DAG and IP3. While IP3 increases intracellular calcium, DAG activates PKC, further contributing to downstream events.Additionally, the molecular chaperone Hsp90 is recruited to the activated VEGFR2 complex, ensuring proper folding, stabilization, and functional integrity of eNOS, while preventing its degradation. The combined activity of Ca2+, CaM, and Hsp90 leads to full eNOS activation, enabling the conversion of molecular oxygen and L-arginine into NO. The generated NO exerts multiple biological functions, including enhancing vascular permeability, inducing vasorelaxation, and maintaining endothelial cell survival (Rybak-Krzyszkowska *et al*., 2023).

II.3.3.2. The Heme Oxygenase Pathway (The HO/CO System)

Heme oxygenase (HO) is the rate-limiting enzyme in heme catabolism, operating in the endoplasmic reticulum to generate equimolar quantities of biliverdin, free iron, and carbon monoxide (CO) (Tenhunen *et al*., 1969). Biliverdin is subsequently converted into bilirubin by

biliverdin reductase, and bilirubin acts as a strong antioxidant. CO functions as a potent vasodilator and also exerts anti-apoptotic effects.

There are two main isoforms of HO:

- HO-2: a constitutive 36 kDa protein highly expressed in the brain, testes, and vascular endothelium.
- HO-1: a 32 kDa inducible protein, widely distributed with higher levels in the liver and spleen.

In mammalian tissues, HO-1 is upregulated by its substrate (heme) and by exposure to heavy metals. Additional inducers include oxidative stress stimuli such as peroxynitrite, modified lipids, hypoxia, hyperoxia, ischemia/reperfusion injury, hyperthermia, and endotoxic shock (Sikorski *et al*., 2004). Through its metabolic products, HO-1 exerts cytoprotective actions by reducing oxidative stress, inflammation, and apoptosis (Dulak *et al*., 2008).

In pre-eclampsia, reduced HO-1 expression leads to enhanced activity of anti-angiogenic mediators sVEGFR-1 and sEng (Cudmore *et al*., 2007) (Figure II.5).

II.3.3.3. The Hydrogen Sulfide Pathway (The CSE/H_2S System)

Hydrogen sulfide (H_2S) is a gaseous signaling mediator with multiple biological functions. It induces vasodilation (Zhao *et al*., 2001), exhibits cytoprotective and anti-inflammatory actions (Zanardo *et al*., 2006), and confers protection against cellular injury caused by reperfusion or severe hypoxia (Blackstone & Roth, 2007). Furthermore, it promotes vascular angiogenesis (Papapetropoulos *et al*., 2009). Endogenous H_2S production is catalyzed by three enzymes: cystathionine-γ-lyase (CSE, also known as Cth), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (MPST), which act on cystathionine, homocysteine, cysteine, and mercaptopyruvate, respectively (Bir & Kevil, 2013). While CBS is highly expressed in the brain (Kery *et al*., 1994), CSE is the predominant source of vascular H_2S (Kabil *et al*., 2011).

Analogous to HO-1, which downregulates sVEGFR-1 and sEng (Cudmore *et al*., 2007), the CSE/H_2S pathway appears to exert similar effects (Wang *et al*., 2013) (Figure II.5).

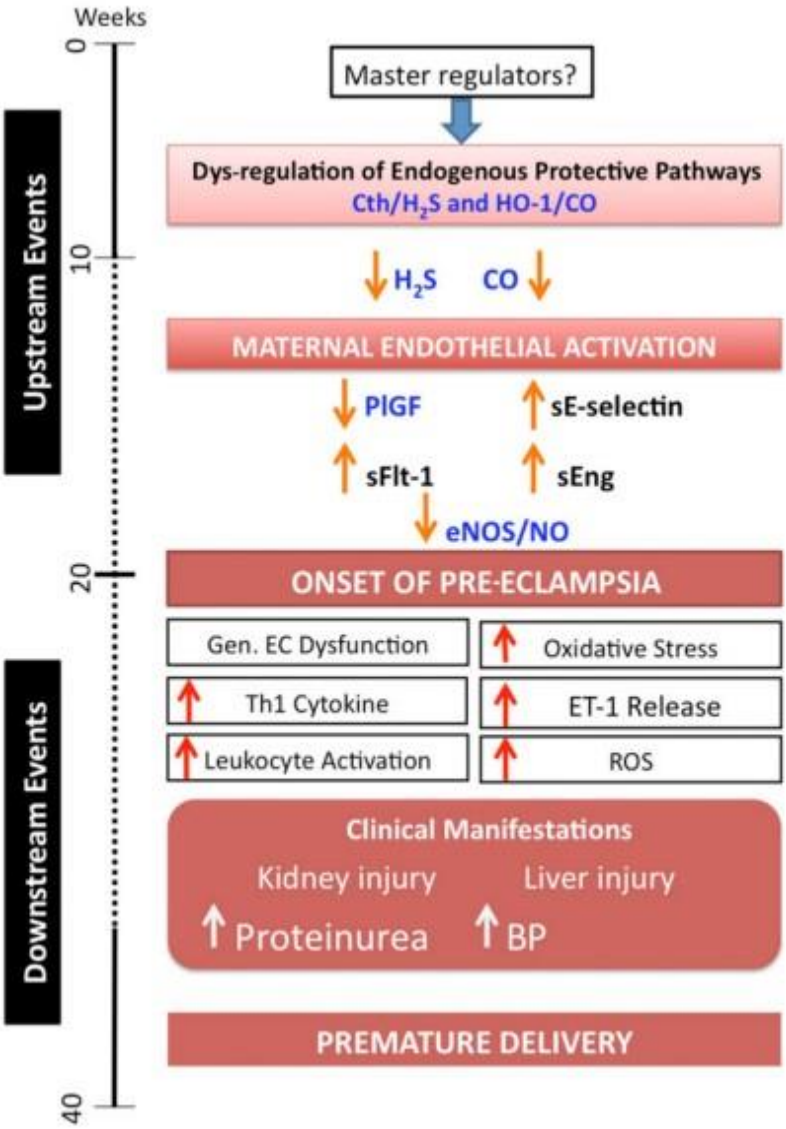

Figure II.5: The "accelerator and brake" model of preeclampsia illustrates the cascade of pathological events driving the disorder. The initiating phase involves dysregulation of endogenous cytoprotective pathways – the "brakes" – including CSE, which produces hydrogen sulfide (H_2S), and HO-1, which generates carbon monoxide (CO). Loss of these protective mechanisms triggers maternal endothelial activation. This is followed by an upregulation of anti-angiogenic mediators – the "accelerator" – such as sVEGFR-1, sEng, and soluble E-selectin, accompanied by a reduction in pro-angiogenic factors like PlGF and eNOS-derived nitric oxide (NO). Collectively, these biochemical alterations result in systemic endothelial dysfunction, renal damage, and increased formation of reactive oxygen species, preceding the clinical onset of preeclampsia. After 20 weeks of gestation, the disorder manifests with hypertension and proteinuria, coinciding with heightened inflammation, as reflected by enhanced production of Th1-type cytokines and elevated ET-1 release (Ahmed & Ramma, 2015).

II.4. Oxidative Stress

Circulating factors in the maternal blood of women with preeclampsia can stimulate oxidative stress within endothelial cells (ECs). These include reactive oxygen species (ROS) derived from neutrophils (oxLDL), agonistic autoantibodies targeting angiotensin receptors (AT1-AA), free fetal hemoglobin (HbF), circulating xanthine oxidase (XO), and pro-inflammatory cytokines such as TNF-α. Within ECs, multiple enzymatic pathways—including the mitochondrial electron transport chain, NADPH oxidases, and cyclooxygenases—contribute to the generation of superoxide (O2•−). Under specific conditions, this process can be accompanied by enhanced Arginase II expression, accumulation of asymmetric dimethylarginine (ADMA), depletion of the cofactor tetrahydrobiopterin (BH4), and uncoupling of endothelial nitric oxide synthase (eNOS). In this uncoupled state, eNOS produces O2•− rather than nitric oxide (•NO). Nitric oxide may subsequently interact with O2•− to form peroxynitrite (ONOO−), a potent oxidant capable of inducing protein nitration and DNA damage, while also inhibiting eNOS activity. Moreover, superoxide-mediated depletion of •NO impairs endothelium-dependent vasodilation. ROS also suppress calcium-activated potassium channels (KCa2.3 and KCa3.1), which are essential for initiating vasodilatory electrical signals (Aouache et al., 2018) (Figure II.6).

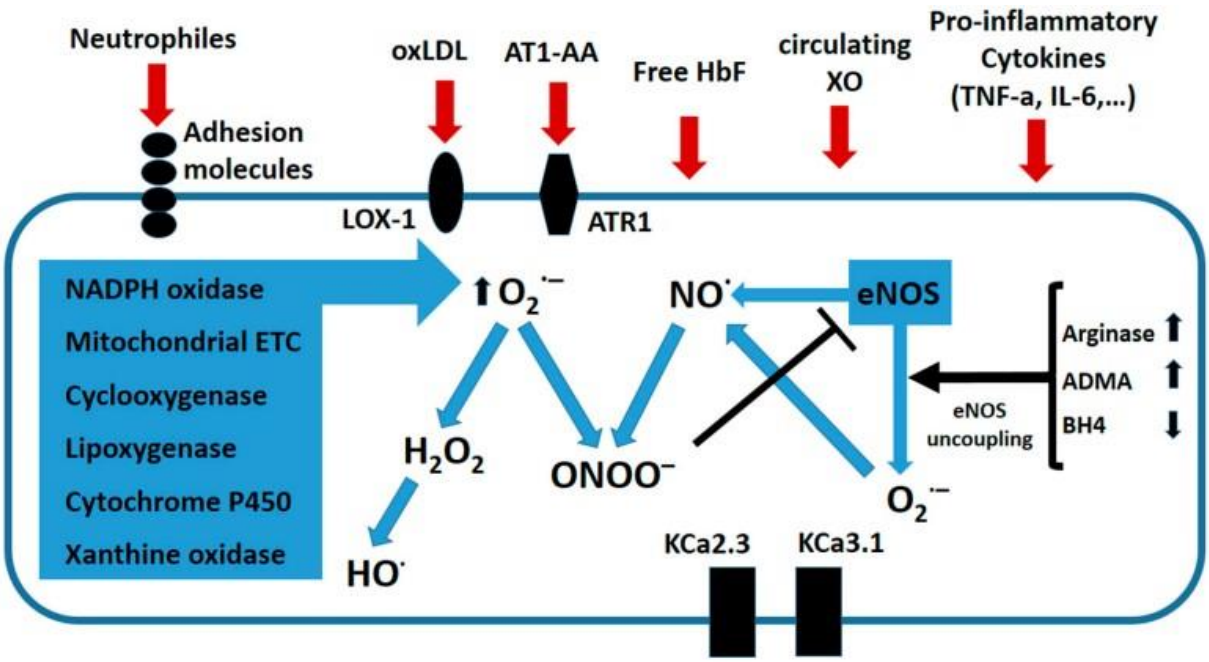

Figure II.6: Key mediators and sources of oxidative stress in preeclamptic endothelium (Aouache *et al.*, 2018).

II.5. Immune and Inflammatory Dysregulation

The systemic inflammatory state observed in preeclampsia is rooted in immunological interactions within the decidua, where maternal immune cells engage directly with trophoblasts at the maternal–fetal interface in placental tissue and secondary lymphoid sites (Staff *et al*., 2014) :

1. In healthy gestation, cytotrophoblasts express HLA-C, HLA-E, and HLA-G, which interact with inhibitory receptors (KIR, CD94/NKGs, ILT-2) on NK cells, thereby preventing NK-mediated damage to placental and fetal tissues (Figure II.1) (Kanagal *et al*., 2014). In PE, reduced HLA-C/KIR interaction increases NK cell activity, promoting placental and fetal injury (Hiby *et al*., 2004). Furthermore, while normal pregnancy involves moderate complement activation, hypertensive pregnancy is marked by elevated complement products (Bb, C3a, C5a) (Lillegard *et al*., 2013).
2. An imbalance in the Th1/Th2 paradigm, characterized by a shift toward the Th1 profile—marked by the secretion of pro-inflammatory cytokines (such as IL-2, IL-6, IL-8, IFN-γ, and TNF-α)—at the expense of Th2 cells, which produce anti-inflammatory cytokines (such as IL-4, IL-10, and IL-13), contributes to the establishment of a systemic inflammatory milieu. This, in turn, underlies the failure of immune tolerance and the emergence of immunoregulatory disturbances in preeclampsia (Raghupathy, 2013).
3. An imbalance in the Th17/Treg cell ratio, characterized by a reduced frequency of Treg cells and elevated levels of Th17 cells secreting the pro-inflammatory cytokine IL-17, promotes the induction of a wide range of cytokines (such as IL-6, IL-8, GM-CSF, and G-CSF), chemokines (including CXCL1 and CXCL10), and metalloproteinases(Kolls & Lindén, 2004; Romagnani, 2008).
4. In preeclampsia, macrophages are frequently associated with a polarization toward the M1 phenotype, characterized by antigen presentation, production of IL-12 and IL-23, as well as the generation of nitric oxide (NO) and reactive oxygen species (ROS), thereby promoting Th1 immune responses. This occurs at the expense of the M2 phenotype, which is distinguished by the secretion of IL-10, IL-6, IL-1Ra, and IDO, conferring anti-inflammatory and immunosuppressive effects; the inhibition of pro-inflammatory cytokines such as IL-12, IL-1β, and IL-8; the support of Th2 responses; and the induction of Treg ($CD4^+CD25^+FoxP3^+$) differentiation through IDO and TGF-β production. Moreover, M2 macrophages attenuate NK cell cytotoxicity via TGF-β signaling, contribute to extracellular matrix remodeling through MMP9 and fibronectin-

1, and promote angiogenesis via EGF and VEGF, in addition to enhancing decidual cell proliferation and invasion through IL-33. These observations underscore the critical importance of maintaining a balanced M1/M2 ratio (Li *et al*., 2020).

II.6. RAAS Alterations in Preeclampsia

Compared with normal pregnancies, women with PE show reduced circulating levels of renin, aldosterone, Ang I, and Ang II. This low-renin profile suggests impaired RAAS regulation (Shoemaker *et al*., 2023). However, they exhibit increased vascular and adrenal responsiveness to Ang II, which exacerbates hypertension and vascular dysfunction characteristic of the disorder (Maamor *et al*., 2024). Ang 1–7, which counteracts Ang II through vasodilation, is frequently dysregulated in PE, amplifying vasoconstriction and hypertension. Experimental findings indicate that impaired placental perfusion enhances local Ang II production, although systemic concentrations remain reduced (Lumbers *et al*., 2019).

Aldosterone also plays an essential role in fluid and sodium balance during pregnancy (Schoenaker *et al*., 2014), and its levels normally rise to meet increased circulatory demands (Jee & Sawal, 2024). In PE, this mechanism is disrupted, with aldosterone concentrations markedly reduced, possibly reflecting decreased plasma volume (Schoenaker *et al*., 2014). In certain cases, high aldosterone secretion paradoxically suppresses renin activity, contributing to disease onset (Artemieva *et al*., 2022). Low-renin hypertension may even point to underlying primary aldosteronism (Schoenaker *et al*., 2014) (Figure II.7).

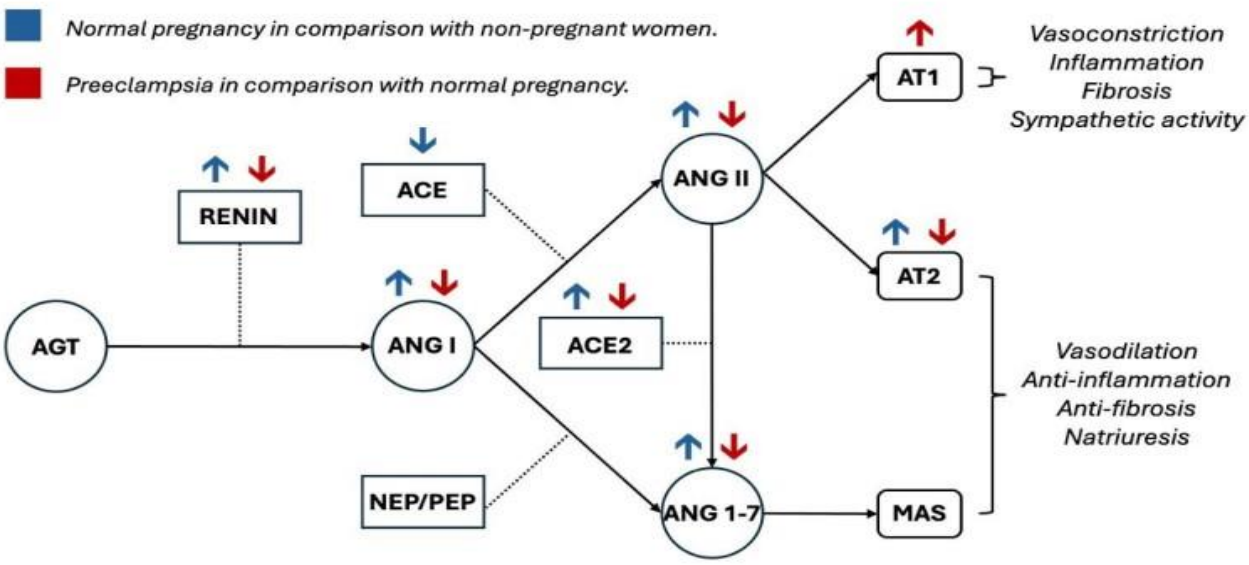

Figure II.7: Alterations in the renin–angiotensin–aldosterone system in non-preeclamptic versus preeclamptic pregnancies (Tsikouras *et al*., 2025).

Chapitre III: Long-term Cardiovascular Consequences in Offspring of Preeclamptic Pregnancies

Chapter III. Long-term Cardiovascular Consequences in Offspring of Preeclamptic Pregnancies

Preeclampsia generates an adverse intrauterine environment characterized by hypertension, hypoxia, and inflammation. As a result, offspring cardiovascular health may be primarily influenced through developmental programming. Consistently, children exposed to maternal preeclampsia show a higher risk of cardiovascular disease in adulthood (Huang *et al*., 2021).

III.1. Cardiovascular Structural and Functional Changes in Offspring of Preeclampsia Pregnancies

III.1.1. Structural Changes

The Rotterdam Periconceptional Cohort Study highlighted macroscopic morphological outcomes in neonates immediately after birth, revealing a reduced umbilical vein area and wall thickness in infants from preeclamptic pregnancies (Herzog *et al*., 2017).

Additionally, neonates of preeclamptic mothers (<48 hours old) exhibited thinner interventricular septa and significantly enlarged lumen diameters in the left and right main coronary arteries, as well as the mitral, tricuspid, aortic, and pulmonic valves (Lin *et al*., 2021).

Children aged 5–8 years born after preeclamptic pregnancies displayed a significantly shorter LV end-diastolic length, indicative of a smaller heart, which may be compensated by a higher heart rate (Fugelseth *et al*., 2011).

In adolescence, offspring exposed to preeclampsia had greater mean relative wall thickness and lower LV end-diastolic volumes compared with children of normotensive pregnancies (Timpka *et al*., 2016).

III.1.2. Functional Changes

Preeclampsia is associated not only with structural cardiovascular changes but also with functional impairments in offspring. Among neonates under 48 hours old, systolic and diastolic blood pressures, fractional shortening, ejection fraction, and cardiac index were elevated in the preeclampsia group after adjusting for birth weight and interactive effects (Lin *et al*., 2021).

Children aged 5–8 years from preeclamptic pregnancies exhibited increased late diastolic velocity (A′ wave) at mitral valve attachments (Fugelseth *et al*., 2011).

In early adolescence (11–12 years), these offspring showed higher systolic and diastolic blood pressures compared with children from normotensive pregnancies, with systolic pressure demonstrating a more pronounced increase (Øglænd *et al*., 2009).

III.2. Underlying Mechanisms of Cardiovascular Alterations in Preeclampsia Offspring

Current knowledge on genetic contributions in preeclampsia offspring is largely derived from animal models. Nair et al used transgenic mice with an endothelial-specific dominant-negative PPARγ mutation (E-V290 M) to examine protection against cardiovascular stress in offspring, particularly males. Their study revealed heightened Ang II sensitivity and endothelial dysfunction in E-V290 M offspring exposed to arginine vasopressin-induced preeclampsia. Activation of the RhoA/Rho kinase pathway and increased ROCK2 expression were implicated in Ang II–mediated endothelial dysfunction in adult offspring of preeclamptic pregnancies (Nair *et al*., 2019).

Induced nitric oxide deficiency led to decreased gene expression associated with arterial smooth muscle contraction (α-actin, SM22α) in newborns, suggesting that nitric oxide is necessary for the regulation of vascular smooth muscle and may contribute to the development of cardiovascular disease later in life (Shvetsova *et al*., 2021).

Hypermethylation in intergenic regions altered expression of imprinted genes DLK1 and MEG3 in human umbilical vein endothelial cells from preeclampsia pregnancies, accompanied by lower nitrite and endothelial growth factor secretion and higher ET-1, potentially promoting endothelial dysfunction and premature CVD (Yu *et al*., 2019).

III.3. Endothelial Dysfunction and Oxidative Stress

VCAM-1 plays a central role in leukocyte adhesion, thrombogenicity, and intravascular coagulation (van der Wal *et al*., 1992). VCAM-1 expression in the umbilical artery endothelium of preeclamptic pregnancies, correlating with maternal systolic blood pressure and enlarged coronary arteries, suggesting VCAM-1 as a pathogenic mediator linking endothelial dysfunction and coronary dilation in offspring. Reduced cord blood NO levels in neonates from preeclamptic pregnancies further support impaired vasodilation and early cardiovascular risk (Deniz *et al*., 2019).

Preeclampsia often results in fetal hypoxia due to reduced placental blood flow, which is manifested by an abnormal increase in reactive oxygen species, thereby indicating placental oxidative stress (Hula *et al*., 2021) (Figure III.1).

III.4. Renal Dysfunction and Cardiovascular Risk in Women with Preeclampsia and Their Offspring

Renal function is crucial for maintaining cardiovascular homeostasis, particularly blood pressure regulation via the renin-angiotensin-aldosterone system. Women with prior preeclampsia exhibit postpartum renal impairment (Reynolds & Herrera, 2020), while offspring of preeclamptic pregnancies show reduced nephron numbers due to low birth weight and prematurity (Wojczakowski *et al*., 2021). This nephron deficit lowers renal filtration, increases circulating blood volume, and contributes to elevated blood pressure. Renal dysfunction, therefore, represents a critical factor when assessing long-term cardiovascular risks in this population (Keller *et al*., 2003).

III.5. Sex Differences

Sexual dimorphism exists in CVD prevalence, pathophysiology, clinical manifestations, and developmental programming (Kamon *et al*., 2022). Males exhibit a more pronounced vascular effect, with the onset of hypertension occurring much earlier than in females (Intapad *et al*., 2013).

III.6. Potential Early Monitoring Indicators for Preeclampsia-Associated CVD Risk

Women with a history of preeclampsia may demonstrate increased left ventricular mass index and diastolic blood pressure, measurable by echocardiography, indicating predisposition to chronic hypertension (Borges *et al*., 2018). Regular follow-up is warranted, particularly when traditional risk factors—such as smoking, obesity, metabolic syndrome, or family history of premature CVD—are present (Mosca *et al*., 2011). Recently, novel markers like AIx at 75 bpm have been introduced, and combining traditional and novel indicators may enhance risk prediction. Pharmacological and lifestyle interventions—including diet, exercise, and smoking cessation—remain essential (Ahmed *et al*., 2014).

MicroRNA profiling provides additional predictive value. The combination of miR-17-5p, miR-29a-3p, and miR-133a-3p effectively identifies at-risk women with prior preeclampsia. For early-onset preeclampsia, miR-1-3p, miR-17-5p, and miR-133a-3p offer superior predictive

performance, whereas miR-29a-3p alone is sufficient for late-onset cases. Women with previous severe preeclampsia may benefit from a panel including miR-17-5p, miR-20b-5p, miR-29a-3p, miR-126-3p, and miR-133a-3p for optimal cardiovascular risk stratification (Hromadnikova *et al*., 2019).

Everything is summarized in (Figure III.1).

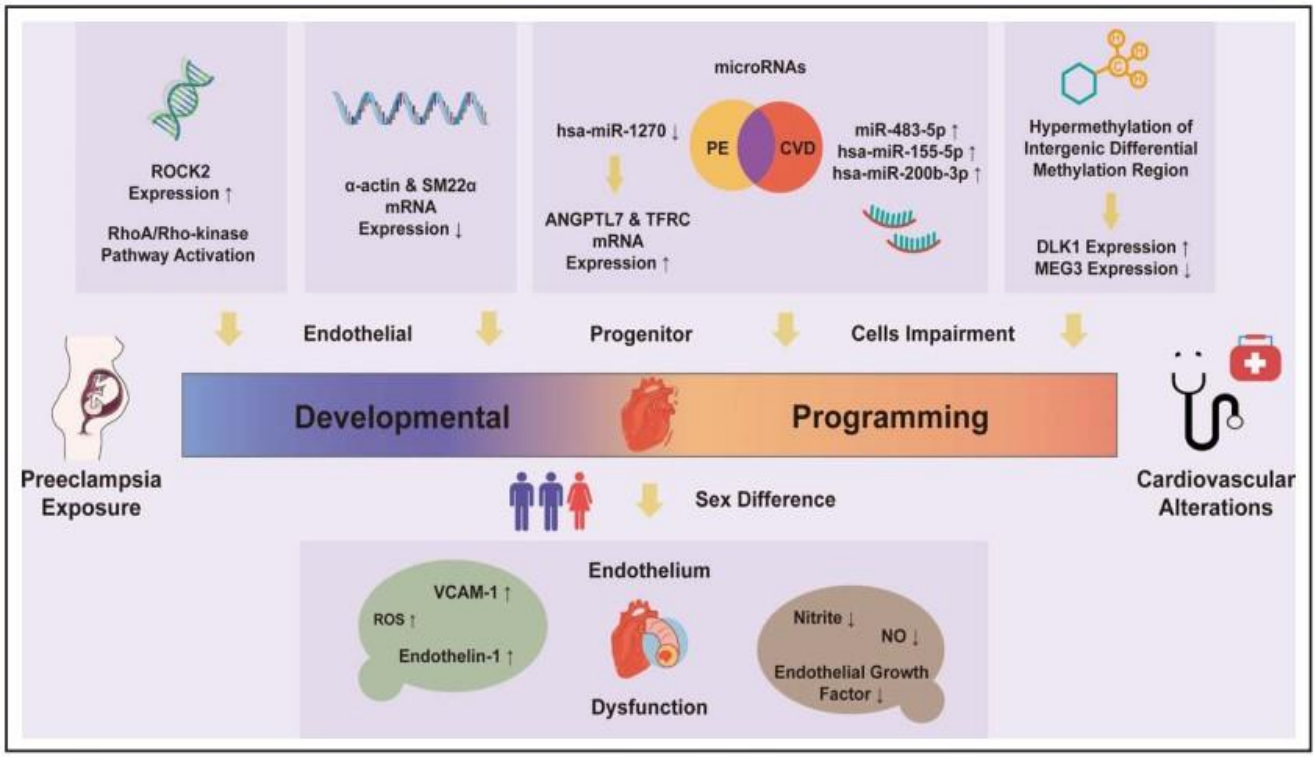

Figure III.1: Mechanistic pathways implicated in long-term cardiovascular changes in the offspring of preeclamptic pregnancies (C. Yang *et al*., 2023).

Chapter IV: Comparative Analysis of Scientific Findings and Research Gaps

Chapter IV. Comparative Analysis of Scientific Findings and Research Gaps

IV.1. Placental Dysfunction and Fetal Programming

Placental dysfunction is central to the pathogenesis of preeclampsia. Deficient remodeling of spiral arteries and insufficient trophoblast invasion result in placental ischemia, which compromises the transfer of oxygen and nutrients to the fetus. Such ischemia is closely linked to intrauterine growth restriction (IUGR), a key risk factor for later cardiovascular and metabolic complications (Redman & Sargent, 2005).

Fetal programming emerges from this inadequate nutrient and oxygen supply, inducing maladaptive cardiovascular alterations that may persist well into adulthood. Consequently, placental function plays a decisive role not only in short-term neonatal health but also in long-term cardiovascular trajectories.

Implications: Strengthening prenatal monitoring and targeted management of placental health may help mitigate risks associated with preeclampsia. Early interventions could improve fetal growth and lessen adverse long-term outcomes.

Limitations: Variability in placental performance and population-specific effects complicate generalization of findings. Moreover, the long-term impacts of placental dysfunction are difficult to assess comprehensively due to ethical and logistical research constraints (Balani *et al*., 2025).

IV.2. Shared Genetic and Environmental Influences

Evidence suggests that preeclampsia has a heritable component, with genetic factors accounting for roughly 31% of its occurrence. Offspring of both affected mothers and fathers exhibit a higher risk of developing preeclampsia and later cardiovascular disease (CVD).[8,13,14] This familial pattern highlights that inherited predispositions, especially toward vascular disorders, may elevate CVD risk independently of direct preeclampsia exposure (Q. Yang et al., 2023).

Beyond genetics, shared lifestyle and environmental influences are significant contributors. Family-wide tendencies toward unhealthy behaviors—such as poor diet, inactivity, and smoking—further increase the risk of both preeclampsia and CVD in children. These additional “second hits” can interact with genetic vulnerabilities, amplifying disease susceptibility across generations (Lu & Hu, 2019).

Implications: Promoting healthier lifestyle habits and educating families about genetic predispositions linked to preeclampsia may reduce the intergenerational burden of CVD. Genetic counseling could also serve as a valuable tool for family risk awareness.

Limitations: The inheritance of preeclampsia is shaped by complex gene–environment interactions that remain incompletely defined. Furthermore, the reliance on observational studies makes it difficult to determine causality (Balani *et al*., 2025).

IV.3. Epigenetics

Epigenetic mechanisms, including DNA methylation and histone modifications, provide a biological bridge linking maternal preeclampsia to offspring health outcomes. These alterations affect gene expression without changing the DNA sequence, thereby influencing fetal growth and later-life disease risk. Maternal nutrition and psychosocial stress during pregnancy are key environmental drivers of such changes, underscoring the role of maternal health in shaping long-term health trajectories in offspring (Marciniak *et al*., 2017). Epigenetic reprogramming may impair stress regulation, metabolic balance, and vascular function, thereby predisposing children to CVD.

Implications: Advancing knowledge on epigenetics could enable preventive interventions, such as improving maternal nutrition, to counteract adverse programming.

Limitations: Research in this area is still emerging, with the multifactorial and dynamic nature of epigenetic regulation complicating the identification of causal pathways. Additionally, whether these changes can be reversed remains uncertain (Balani *et al*., 2025).

IV.4. Inflammation

Persistent inflammation is a defining feature of preeclampsia, where placental ischemia initiates a cascade involving cytokine release and immune activation. Such an inflammatory milieu can disrupt fetal growth, induce vascular injury, and foster atherosclerotic changes in offspring. Elevated pro-inflammatory cytokines may contribute to long-term alterations in vascular integrity, predisposing children to hypertension and cardiovascular disorders (Q. Yang et al., 2023).

The combined influence of inflammation and oxidative stress further complicates the intrauterine environment, intensifying risks for the fetus.

Implications: Strategies that lower inflammation during pregnancy, including lifestyle interventions or targeted therapeutics, may support fetal development and cardiovascular outcomes.

Limitations: Inflammatory responses vary widely across individuals, hindering the design of universal interventions. The timing and persistence of inflammation's effects on fetal growth also remain insufficiently understood (Balani *et al*., 2025).

IV.5. Angiogenic Imbalance and Endothelial Dysfunction

Preeclampsia is marked by disruption in angiogenic signaling, with elevated levels of anti-angiogenic proteins such as sFlt-1 and sEng. These inhibit pro-angiogenic mediators like VEGF, culminating in endothelial dysfunction (Rana *et al*., 2022).

As a precursor to atherosclerosis, endothelial dysfunction has major consequences for offspring cardiovascular health. Impairments in blood and nutrient flow to the fetus may increase the likelihood of long-term vascular complications. Experimental studies targeting these pathways have shown promise in preventing offspring hypertension, emphasizing the need for continued investigation (Rodríguez-Rodríguez *et al*., 2017).

Implications: Monitoring angiogenic balance throughout pregnancy could represent a valuable intervention point to mitigate future cardiovascular risk in offspring.

Limitations: Research on angiogenic mediators is still emerging, and translating preclinical insights into practical treatments is challenging. Additionally, heterogeneity in patient responses complicates the establishment of standardized therapies (Balani *et al*., 2025).

IV.6. Oxidative Stress

Oxidative stress from excess ROS production during preeclampsia endangers fetal development. Elevated ROS can damage the placenta, restrict fetal growth, and impair lipid metabolism and insulin sensitivity, thereby increasing vulnerability to metabolic and cardiovascular disease later in life (Alexander *et al*., 2015).

The interplay of oxidative stress with inflammation and endothelial dysfunction forms a self-perpetuating cycle, compounding maternal and fetal health risks.

Implications: Reducing oxidative stress via antioxidants or lifestyle modification may safeguard fetal growth and cardiovascular outcomes.

Limitations: Individual differences in oxidative stress responses complicate therapy design, and the long-term safety and effectiveness of antioxidant use in pregnancy remain uncertain (Balani *et al*., 2025).

IV.7. Dysregulation of the RAAS

In pregnancy, disruption of the renin–angiotensin–aldosterone system (RAAS) plays a critical role in preeclampsia. Upregulation of ACE in fetal endothelial cells promotes excessive Ang II production, impairing uteroplacental circulation and placental development (Gathiram & Moodley, 2020). Such dysregulation has been associated with sex-specific hypertension risk, reflecting heightened Ang II sensitivity in both male and female offspring. Alterations in RAAS also contribute to insulin resistance and nephron deficits, intensifying cardiovascular risk profiles in exposed children.

Implications: Insights into RAAS dysregulation may guide therapeutic approaches for maternal hypertension and help reduce long-term cardiovascular consequences in offspring.

Limitations: The intricate nature of RAAS and its hormonal cross-talk complicates both research and clinical translation, while variability in therapeutic response remains a challenge (Balani *et al*., 2025).

Everything is summarized in (Figure IV.1).

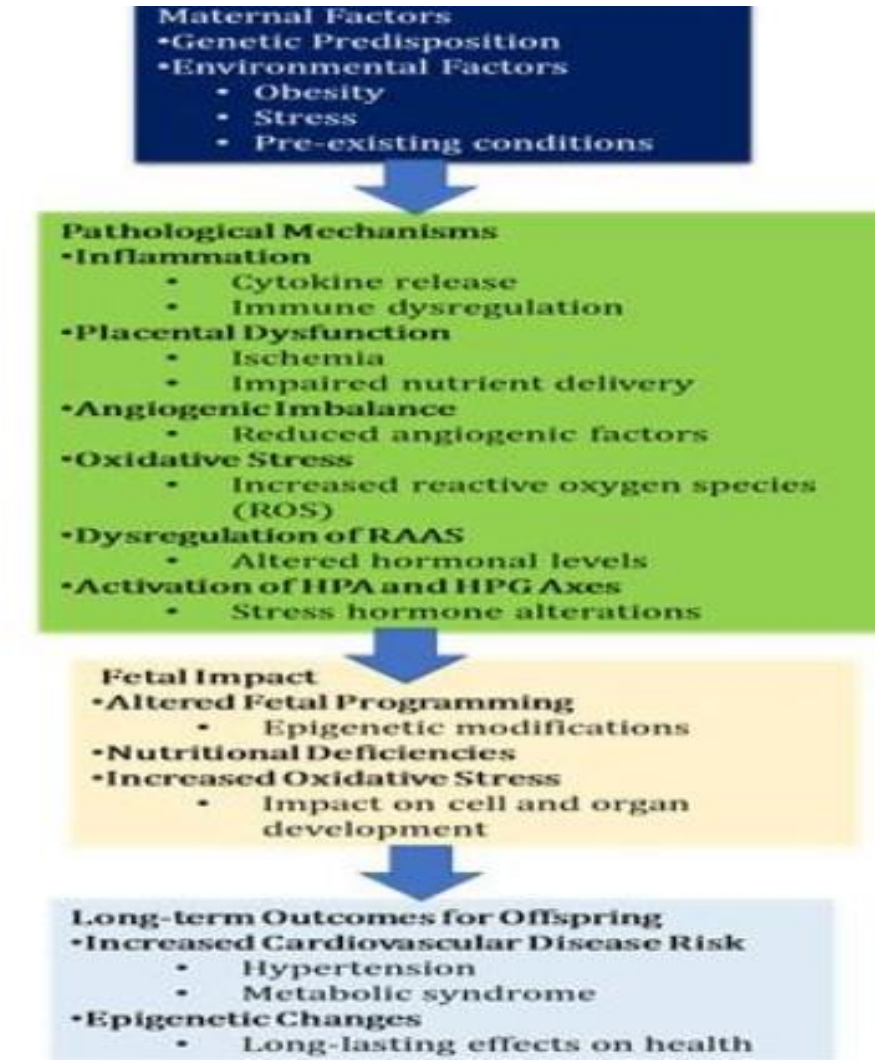

Figure IV.1: Maternal, Pathophysiological, and Fetal Mechanisms Linking Preeclampsia to Long-Term Cardiovascular Risk in Offspring (Balani *et al*., 2025).

Conclusion

Conclusion

Preeclampsia remains one of the most significant complications of pregnancy, with profound short- and long-term consequences for both mother and child. This thesis has examined its biochemical and molecular foundations, highlighting the interplay between maternal predisposition, placental dysfunction, angiogenic imbalance, oxidative stress, immune dysregulation, and endocrine disturbances. Collectively, these mechanisms create a hostile intrauterine environment that not only endangers maternal health but also exerts lasting effects on the offspring.

A central theme emerging from the evidence is that preeclampsia initiates a process of fetal programming. Epigenetic modifications, altered gene expression, impaired nutrient delivery, and sustained oxidative stress shape the developmental trajectory of the fetus in ways that persist beyond birth. Children born to preeclamptic pregnancies exhibit measurable changes in vascular structure and function, including endothelial dysfunction, arterial stiffness, and a heightened susceptibility to hypertension and metabolic syndrome. These findings underscore the notion that preeclampsia is not limited to a transient disorder of pregnancy but represents a disease with intergenerational consequences.

From a clinical perspective, these insights call for a paradigm shift in how preeclampsia is managed. Beyond acute obstetric care, attention must be directed toward the long-term cardiovascular health of offspring, necessitating strategies for early screening, preventive interventions, and lifestyle modifications that mitigate future disease risk. Public health policies must also consider preeclampsia as an early-life determinant of cardiovascular disease, with implications for resource allocation, maternal health programs, and preventive cardiology.

At the same time, substantial research gaps remain. The exact molecular mediators linking maternal preeclampsia to offspring cardiovascular outcomes are incompletely defined, particularly in humans. Most epigenetic evidence is derived from cord blood and placental tissue, requiring further validation in longitudinal studies. Similarly, sex-specific differences, the influence of maternal comorbidities, and the potential for targeted therapeutic interventions remain areas of active investigation.

In conclusion, preeclampsia represents a unique intersection of obstetrics, cardiovascular medicine, and developmental biology. By elucidating its biochemical and molecular mechanisms and tracing their impact on offspring health, this work contributes to a deeper understanding of how an adverse intrauterine environment shapes lifelong disease risk. The challenge moving

forward lies in translating this knowledge into early detection tools, preventive strategies, and clinical interventions that improve outcomes not only for mothers but also for future generations.

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Appendices

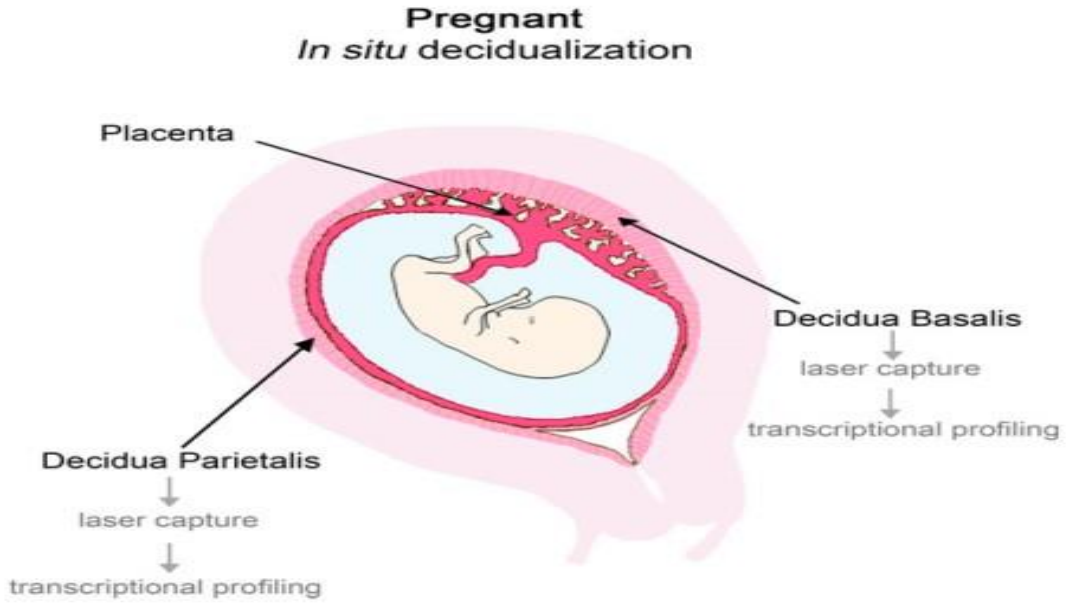
Appendices

Appendix A: The method for measuring hypertension (Maxwell *et al*., 1982; Paulo, 2010).

hypertension: blood pressure ≥ 140/90 mmHg measured after rest, with the patient seated and using an appropriate cuff. The first Korotkoff sound defines systolic pressure, and the fifth sound (disappearance) defines diastolic pressure. If sounds persist, the muffling point is used for diastolic pressure. A correction table should be applied if the cuff size is unsuitable (Program, 2000).

Arm circunference: cm	Systolic BP: mmHg	Diastolic BP: mmHg
20	+11	+7
22	+9	+6
24	+7	+4
26	+5	+3
28	*+3*	+2
30	0	0
32	-2	-1
34	-4	-3
36	-6	-4
38	-8	-6
40	-10	-7
42	-12	-9
44	-14	-10
46	-16	-11

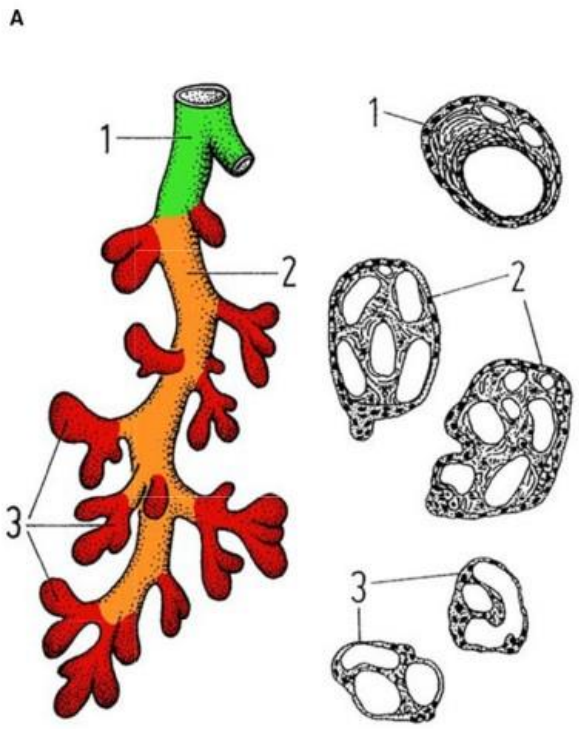
48	-18	-13
50	-21	-14



Appendix B: Embryo and Placental Positioning within the Uterus (the decidua basalis (DB) and decidua parietalis (DP)) (Garrido-Gomez *et al*., 2017).

Appendix C: (A) Diagram of the villous tree structure including (Turowski & Vogel, 2018):

1. Stem villus.
2. Peripheral-type intermediate villus.
3. Terminal villus.



ملخص

يُعَدّ تسمم الحمل اضطراباً حملياً معقداً يتميز بارتفاع ضغط الدم وينشأ نتيجة اختلالات بيوكيميائية وجزيئية على مستوى الواجهة المشيمية–الأمومية. وتشمل الآليات المركزية الإجهاد التأكسدي، والخلل البطاني، واختلال التوازن في العوامل الوعائية، حيث يتمثل ذلك في تغير مستويات VEGF و PlGFو .sFlt-1كما يُسهم اضطراب نظام الرينين–أنجيوتنسين–ألدوستيرون، وزيادة إنتاج أنواع الأكسجين التفاعلية، وارتفاع الوسائط الالتهابية مثل VCAM-1 في تفاقم الضرر الوعائي وانخفاض توافر أوكسيد النيتريك. تؤدي هذه العمليات الفيزيوباثولوجية إلى نقص التأكسج المشيمي وتنشيط بطاني جهازي، وهو ما يفسر السمات السريرية لتسمم الحمل. والأهم من ذلك، أن البيئة الرحمية غير المواتية تترك آثاراً طويلة الأمد على النسل؛ إذ يرتبط نقص أوكسيد النيتريك في دم الحبل السري، والتغيرات في التعبير الجيني للجزيئات اللاصقة، والتعديلات فوق الجينية بزيادة القابلية للإصابة المبكرة بالاختلال الوعائي وارتفاع خطر الأمراض القلبية الوعائية لاحقاً. وعليه، يُمثل تسمم الحمل ليس فقط مضاعفة حمليّة بل محدداً أساسياً للصحة القلبية الوعائية عبر الأجيال، مما يبرز الحاجة إلى استراتيجيات موجهة للتقليل من عواقبه طويلة المدى على نسل الأمهات المصابات.

Résumé

La prééclampsie est un trouble hypertensif complexe de la grossesse, résultant de perturbations biochimiques et moléculaires à l'interface materno-placentaire. Les mécanismes centraux incluent le stress oxydatif, la dysfonction endothéliale et le déséquilibre angiogénique, caractérisé par des niveaux altérés de VEGF, PlGF et sFlt-1. La dérégulation du système rénine–angiotensine–aldostérone, l'augmentation de la production d'espèces réactives de l'oxygène et la libération accrue de médiateurs pro-inflammatoires tels que VCAM-1 contribuent davantage aux lésions vasculaires et à la diminution de la biodisponibilité du monoxyde d'azote. Ces processus physiopathologiques entraînent une hypoxie placentaire et une activation endothéliale systémique, établissant ainsi les manifestations cliniques de la prééclampsie. Plus encore, l'environnement intra-utérin défavorable exerce des effets durables sur la descendance. La réduction du NO dans le sang du cordon, l'expression anormale des molécules d'adhésion et les modifications épigénétiques prédisposent les nouveau-nés à une dysfonction vasculaire précoce et à un risque cardiovasculaire accru au cours de la vie. Ainsi, la prééclampsie représente non seulement une complication maternelle mais aussi un déterminant de la santé cardiovasculaire intergénérationnelle, soulignant la nécessité de stratégies ciblées visant à réduire ses conséquences à long terme chez la descendance.

Abstract

Preeclampsia is a complex hypertensive disorder of pregnancy driven by biochemical and molecular disruptions at the maternal–placental interface. Central mechanisms include oxidative stress, endothelial dysfunction, and angiogenic imbalance, characterized by altered levels of VEGF, PlGF, and sFlt-1. Dysregulation of the renin–angiotensin–aldosterone system, increased production of reactive oxygen species, and pro-inflammatory mediators such as VCAM-1 further contribute to vascular injury and impaired nitric oxide bioavailability. These pathophysiological processes lead to placental hypoxia and systemic endothelial activation, establishing the clinical features of preeclampsia. Importantly, the adverse intrauterine environment has lasting consequences for offspring. Reduced cord blood NO, abnormal expression of adhesion molecules, and epigenetic modifications collectively predispose neonates to early vascular dysfunction and heightened cardiovascular risk later in life. Thus, preeclampsia represents not only a maternal complication but also a determinant of intergenerational cardiovascular health, underscoring the need for targeted strategies to mitigate long-term outcomes in affected offspring.