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# An examination on the increased prevalence of preeclampsia in people undergoing assisted reproductive treatments

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**AN EXAMINATION ON THE INCREASED PREVALENCE OF  
PREECLAMPSIA IN PEOPLE UNDERGOING ASSISTED REPRODUCTIVE  
TREATMENTS**

by

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B.A., The University of Chicago, 2019

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**ABSTRACT**

Preeclampsia (PE), a leading cause of maternal and fetal morbidity and mortality, affects approximately 4-5% of pregnancies and is characterized by the presence of new-onset hypertension, and frequently proteinuria, after 20 weeks gestation. Individuals diagnosed with preeclampsia have been known to suffer from post-pregnancy complications, like cardiovascular disease, and their risk of developing preeclampsia in a subsequent pregnancy also increases. Although the etiology of this disease is not entirely known, research into potential causes have shown that preeclamptic patients have uterine environments that show excessive oxidative stress, inflammation, an imbalance in the levels of factors, proteins, and immune cells necessary for gestation. In much of the research, the maternal immune system and immune response emerges as a principal cause for placental and endothelial dysfunction, both of which can present in preeclampsia. As studies into the etiology of PE advances, the propensity for particular gestations to present with the disease, such as pregnancies conceived via assisted reproductive technologies (ART), is regularly scrutinized by researchers who aim to better understand this pathology. As more individuals look toward reproductive technologies to aid in conception, insight into the possible obstetric complications faced by patients of ART becomes more relevant. An analysis on how and why ART pregnancies have an increased

risk of developing this disease further elucidates the effects of an abnormal uterine environment and aberrant maternal immune response on placentation and endothelial remodeling. With ART procedures that utilize frozen embryos or donated gametes showing higher rates of PE—and with the use of these ART protocols rising in popularity worldwide—an awareness and knowledge on the disorders that may afflict ART gestations, especially hypertensive disorders of pregnancy, is necessary for both the advancement of this medical technology and for the general understanding of a pathology as complex as preeclampsia.

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## LIST OF ABBREVIATIONS

APC	antigen presenting cell
AREG	amphiregulin
ART	assisted reproductive technology
Bcl-2	B-cell lymphoma/leukemia
BTC	betacellium
CL	corpus luteum
CT	cytotrophoblast
CVD	cardiovascular disease
DAMP	damage-associated molecular patterns
DC	dendritic cell
dN	decidual neutrophil
E2	estradiol
ECM	extracellular matrix
EGF	epidermal growth factor
Eng	endoglin
EREG	epiregulin
ET-1	endothelin-1
EVT	extravillous trophoblast
FET	frozen/fresh embryo transfer
FGR	fetal growth restriction
GM-CSF	granulocyte-macrophage colony-stimulating factor

HBEGF	heparin-binding EGF-like growth factor
hCG	human chorionic gonadotropin hormone
HELLP	hemolysis, elevated liver enzymes, and low platelets
HGF	hepatocyte growth factor
HIF	hypoxia inducing factor
HLA	human leukocyte antigen
HO	heme oxygenase
HUtMvEC	human uterine microvascular endothelial cells
ICSI	intracytoplasmic sperm injection
IFN $\gamma$	interferon gamma
IGF	insulin growth-like factor
IPD	ischemic placental disease
IUI	intrauterine insemination
IVF	in vitro fertilization
KIR	killer cell immunoglobulin-like receptor
LPS	lipopolysaccharide
M $\phi$	macrophage
MBRN	Medical Birth Registry of Norway
M-CSF	macrophage colony-stimulating factor
MHC	major histocompatibility complex
MIF	migration inhibitory factor
mROS	mitochondrial-derived ROS

mtDNA	mitochondrial DNA
NET	neutrophil extracellular trap
NK	natural killer cell
NO	nitric oxide
OD	oocyte donation
OR	oocyte recipient
PAMP	pathogen-associated molecular patterns
PE	preeclampsia
PGE	prostaglandin E
PGI <sub>2</sub>	prostacyclin
PlGF	placental growth factor
ROS	reactive oxygen species
SART	Society for Assisted Reproductive Technology
SCT	syncytiotrophoblast
SD	sperm donation
SET	single-embryo transfer
sEng	soluble endoglin
sFlt-1	fms-like tyrosine kinase
SpA	spiral artery
T1	first trimester (of gestation)
T2	second trimester (of gestation)
TGF- $\alpha$	transforming growth factor- $\alpha$

TGF- $\beta$	transforming growth factor beta
Th1	T helper type-1 cell
Th2	T helper type-2 cell
Th17	T helper type-17 cell
TNF	tumor necrosis factor
TNF- $\alpha$	tumor necrosis factor alpha
Treg	T regulatory cells
uDC	uterine dendritic cell
uILC	uterine innate lymphoid cell
uNK	uterine Natural Killer cell
VEGF	vascular endothelial growth factor
WGS	whole-genome shotgun

## INTRODUCTION

### A Brief History of Preeclampsia

Preeclampsia (PE) is a pathology of pregnancy that affects anywhere between 4-5% of pregnancies worldwide and is considered a leading cause of maternal and fetal morbidity and mortality [1]. Currently, preeclampsia is classified as a hypertensive disorder of pregnancy in which new-onset hypertension arises in previously normotensive women after 20 weeks gestation, with the second diagnostic symptom of proteinuria. The study of hypertension of pregnancy has been recognized and studied by physicians as early as the 17<sup>th</sup> century, along with the rise of the practice of obstetrics. Although preeclampsia was not recognized as its own pathology until the 19<sup>th</sup> century, early identification of symptoms such as inflammation, suppressed lochial flow, and convulsions were seen by 17<sup>th</sup> century physicians in some pregnancies, leading to the 18<sup>th</sup> century differentiation of “epilepsy of the pregnant uterus,” or eclampsia, that is characterized by the propensity for seizures during pregnancy that cease once a woman is postpartum [2].

As the field of obstetrics advanced, theories arose in the 19<sup>th</sup> century to explain some of the symptoms subsequently attributed to preeclampsia/eclampsia. Some of the earliest theories aimed to explain why pregnant women seized either during pregnancy or during labor. In 1821, Dr. Thomas Denman attributed said seizures with a rise in the pressure being exerted on the descending blood vessels by the expanding uterus that could lead to the regurgitation of blood back to the head, thus causing a buildup of blood in the cerebral vasculature resulting in convulsions. Decades later, Dr. William Tyler

Smith challenged Dr. Denman's theory by suggesting that if the rise in cerebral blood pressure caused pregnant women's convulsions, there should be more cases in which women seize during labor due to the contractions that arise in the second stage of labor that interfere with maternal blood flow. Instead, Dr. Smith proposed 5 potential theories as to why seizing may occur: (1) excess mechanical/emotional stimuli to the spinal center; (2) "bloodletting," or the withdrawal of a person's blood to alleviate/cure an illness; (3) variations in physical environment, like weather, temperature, or elevation; (4) irritation of the uterus, uterine passages, intestines or stomach; and (5) "toxic" elements [2]. Although both physicians' theories do not entirely align with the modern hypotheses on the etiology of preeclampsia/eclampsia, they provided a foundation for the eventual refinement of the signs and symptoms for preeclampsia throughout the 19<sup>th</sup> century.

### **The Modern Perception of PE**

Now, prevalent theories of the 20<sup>th</sup> and 21<sup>st</sup> centuries began to focus on various ideas that fuel modern research into the still unknown etiology of preeclampsia. Determinants for PE include familial history, genetic predisposition, duration of sexual cohabitation, maternal smoking, number of pregnancies, maternal age, previous or ongoing maternal medical condition, or the use of *in vitro* or other fertility treatments. Diagnostic factors for mild and severe preeclampsia are outlined in **Table 1**. There is an increase in systolic blood pressure to  $\geq 140$  mmHg or in diastolic blood pressure to  $\geq 90$  mmHg in individuals who were normotensive prior to conceiving [1]. PE can also be classified by the time it was diagnosed during gestation. Early-onset PE is defined by

**Table 1: Diagnostic Factors of Mild and Severe Preeclampsia.** Taken from (Ankichetty et al., 2013) [39].

<b>Parameters</b>	<b>Mild</b>	<b>Severe</b>
Clinical parameters		
Systolic blood pressure (mmHg)	140-160	≥160
Diastolic blood pressure (mmHg)	90-110	≥110
Headache	Absent	Present
Visual disturbances	Absent	Present
Epigastric pain	Absent	Present
Laboratory parameters		
Urinary output	>500 ml/24 h	≤500 ml/24 h
Urinary protein	<5 g/24 h	≥5 g/24 h
Urinary dipstick	1+/2+	3+/4+

premature delivery prior to 34 weeks gestation and has a higher rate of fetal growth restriction (FGR). Late-onset PE is characterized by delivery after 34 weeks gestation where the neonates generally do not show signs of being affected by FGR. Patients with late-onset PE are more likely to be affected with HELLP (hemolysis, elevated liver enzymes, and low platelets) and eclampsia, a severe consequence of PE distinguished by the presence of seizures [3].

Current research on PE now focuses on topics such as abnormal placental physiology, endothelial disorders, and a maternal immunological response as prospective causes of preeclampsia. Preeclampsia is commonly divided into two stages: the placental stage and maternal syndrome. Stage one—the placental stage—suggests that preeclampsia originates in the placenta with common pathologic findings including atherosclerosis, fibrin deposition and infarcts, and narrowing of the arterial vasculature. Stage two—maternal syndrome—is characterized by placental ischemia and hypoxia in which persons with preeclampsia have an increased predisposition for placental infarcts, indicating potential placental hypoperfusion and ischemia [1, 4]. The two-stage model of PE has been refined

as various theories have been investigated. Physicians and researchers have had findings on the role of various leukocytes, trophoblast-derived markers associated with PE (i.e., anti-angiogenic pathway), ischemia-reperfusion injury and placental oxidative stress. As research into the etiology of PE continues, we see certain theories being pushed towards the forefront of various studies as more evidence is discovered on the pathophysiology of PE [3].

### **PE and Assisted Reproductive Technologies**

Despite the plethora of possible determinants for preeclampsia, it has been shown that the risk for developing preeclampsia/eclampsia is higher in people conceiving with assisted reproductive technologies, like *in vitro* fertilization (IVF). Ischemic placental disease (IPD)–preeclampsia, placental abruption, and/or small for gestational age fetus–contributes to more than half of the medically induced deliveries performed before 35 weeks gestation, and to about half of preterm births. IPD is more common in IVF pregnancies when compared to spontaneous conceptions, or conceptions without the use of assisted reproductive technology (ART). Moreover, preeclampsia is more likely to complicate IVF pregnancies than non-IVF pregnancies [5]. IVF procedures–which include IVF, intracytoplasmic sperm injection (ICSI), and fresh or frozen embryo transfer (FET)–that lead to viable pregnancies have at least a two-fold higher risk of developing preeclampsia, with different IVF treatments having varying propensities for the development of IPD [6].

A leading theory as to the increased propensity of developing preeclampsia in IVF pregnancies stems from the proposition that there is an increased risk of abnormal placentation and/or implantation, alongside an abnormal maternal immune response. Maternal immune tolerance is vital in pregnancies with a semi-allogeneic fetus [5, 7]. This brings into question how various factors can affect patients receiving ART treatments. For instance, how does the use of donor or autologous oocytes—or the use of donor sperm— affect the maternal immune response? Further, on average, women considering or undergoing IVF treatments are older and the risk for developing preeclampsia increases significantly in patients over 40. Studies have also shown that pregnancies resulting from oocyte donation (OD) have quadruple the probability of developing PE when compared to spontaneous pregnancies, despite adjusting for the patient's age [7].

Studies on IVF (both oocyte recipient and autologous) versus spontaneous pregnancies attempt to examine why the occurrence of PE is heightened in pregnancies conceived via ART. In the case of IVF, the leading theory centers on a potentially decreased maternal immune tolerance and response towards the fetus, especially in the case of oocyte recipient (OR) IVF gestations [6, 7]. In the attempt to better PE diagnostics, some researchers have aimed to determine biomarkers for the detection of PE that could arise due to a maternal immune response to placental factors and proteins [8, 9]. Although this is only one theory, the examination of the maternal immune response in IVF patients and their increased risk for PE is a topic worth exploring as a

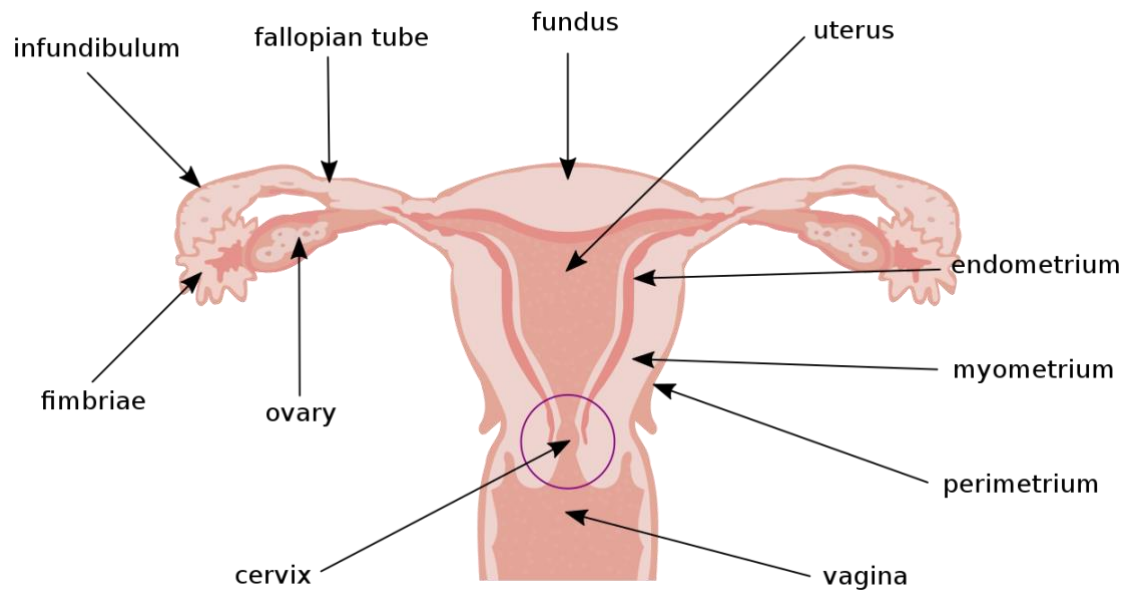
means to further understand both the etiology of this particular pathology of pregnancy, and the particular risks faced by women undergoing fertility treatments.

## IMPLANTATION AND PLACENTATION IN HEALTHY PREGNANCY

### The Inflammatory Stages of Pregnancy

Pregnancy fluctuates through a series of pro-inflammatory and anti-inflammatory stages aimed at ensuring proper implantation and placentation take place to support the growth of the fetus. At the start of gestation there is a pro-inflammatory stage that promotes implantation and placentation. Here we see the active breakdown of the decidua and decidual reconstruction initiated by invading trophoblasts with factors and cytokines such as interleukin-6 (IL-6), IL-8, IL-15, GM-CSF, CXCL1, CCL4, and tumor necrosis factor (TNF) characterizing the pro-inflammatory environment. A pro-inflammatory environment is crucial in encouraging the adhesion and attachment of the blastocyst to the uterine wall by stimulating the expression of adhesion molecules on the surface of the epithelial cells at the uterine lumen. After the blastocyst implants into the decidua, the environment pivots to an anti-inflammatory environment to promote fetal growth and development. This stage is the longest immunological stage during gestation. There needs to be a symbiotic relationship between the mother, fetus, and placenta to support fetal growth. In this stage, several maternal leukocytes play vital roles in cultivating the proper uterine microenvironment to help sustain the pregnancy. The last stage is again a pro-inflammatory stage as the mother prepares for the delivery of the fetus. Once fetal development is complete, the pro-inflammatory nuclear factor- $\kappa$ B signaling pathway initiates labor and delivery. An influx of immune cells to the myometrium are then able to help stimulate the contraction of the uterus, leading to delivery of the baby and the

separation of the placenta [10]. A successful pregnancy should be able to maintain the necessary leukocytic and protein/factor levels at each stage of gestation.

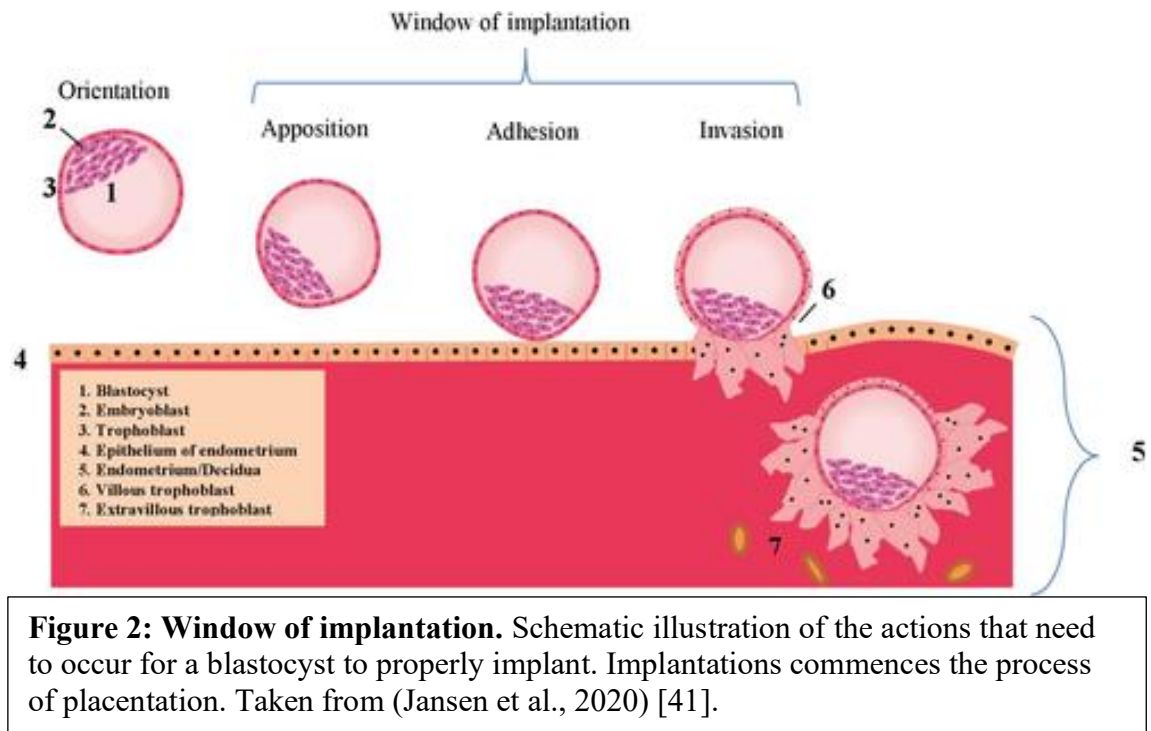


**Figure 1: Female Reproductive System.** An illustration of the female reproductive system with labeled organs and uterine layers. Taken from (National Cancer Institute et al., 2021) [40].

### Implantation

PE is a pathology that requires the presence of a placenta [3]. To reach the events of placentation, the fertilized egg must travel from the fallopian tubes to the uterus (**Figure 1**), where the mass of cells, now called the blastocyst, must implant into the uterine wall to ensure the continuation of the pregnancy (**Figure 2**). The human blastocyst is composed of two separate masses of cells, derived from two distinct lineages. The inner cell mass will become the future embryo, whereas the outer cell mass—also known as the trophoblast—will develop into the future placenta. Implantation is initiated when the trophoblast cells, cells from the outer layer of the

blastocyst, penetrate the epithelial lining of the endometrium, now referred to as the decidua [10]. The decidua is a specialized uterine mucosa originating from the endometrium and modified under the influence of the hormone progesterone. It forms in preparation for blastocyst implantation, and in the case of pregnancy it will be shed after birth. If no implantation occurs it will be shed after every menstrual cycle. The decidua is necessary for implantation and the formation of the placenta [11].



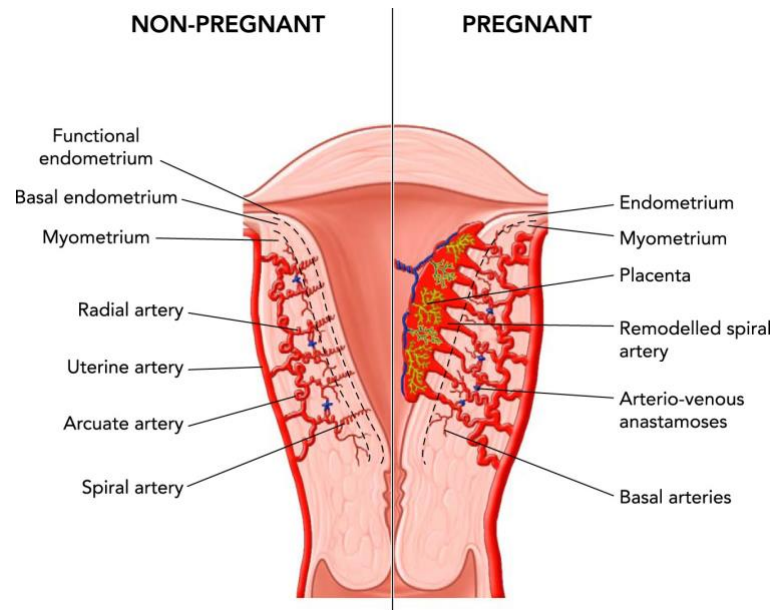
### Placentation and Spiral Artery Remodeling

Placentation relies on the cells of the trophoblast to differentiate and invade the decidua successfully in order to allow for the formation and development of the placenta. A healthy placenta is essential for a normal pregnancy as it is the sole site of nutrient, gas, and waste exchange between the gestational carrier and fetus [12].

Trophoblast cells have two cell populations: cytotrophoblasts (CTs) and

syncytiotrophoblasts (SCTs). Cytotrophoblasts are undifferentiated trophoctoderm cells that lie closer to the inner cell mass. These villous cytotrophoblasts are highly proliferative and can differentiate into outer layer syncytiotrophoblasts, which provide a barrier between the fetal compartment and maternal blood, or extravillous trophoblasts (EVTs). As trophoblasts begin invading the decidua, from the trophoctoderm layer emerges the syncytium. CTs then proliferate to form villi, large finger-like projections, that then penetrate the entire depth of the syncytium. The villi will subsequently become filled with mesenchyme, undifferentiated cells from embryonic connective tissue, that will form the fetal blood vessels that will connect to the fetal circulation via the umbilical cord. The intervillous space can then become bathed in maternal blood. The CTs at the tips of the villi will proliferate and stratify, forming cell columns that are only breached by channels carrying maternal blood toward/away the placenta. The trophoblast cells in these structures are EVT [13]. EVT physically anchor the placenta to the decidua and are vital in the next step of placentation, spiral artery remodeling [12].

Remodeling of the uterine spiral arteries (SpAs) is significantly influenced by the invasion of trophoblast cells into the decidua. Proper placental perfusion allows for the exchange of nutrients and oxygen to the developing fetus. Placentation requires a process of 'reendothelialization,' where the uterine SpAs remodel into larger diameter vessels with low resistance to establish adequate blood supply to the placenta [4, 18]. The SpA remodeling process consists of the loss of the extracellular matrix (ECM), vascular endothelium, and vascular smooth muscle cells. As EVT migrate and invade the uterine arteries they replace the vascular smooth muscle cells, endothelial cells, and elastic



**Figure 3: Non-Pregnant and Pregnant Vasculature.** An illustration of the anatomy of the maternal vasculature in the pregnant and non-pregnant state. In the pregnant state, the spiral arteries are no longer as tightly bound in their characteristic spiral shape as they get remodeled to accommodate the increased uteroplacental blood flow. Taken from (James et al., 2017) [38].

lamina. These changes allow for the enlargement of the vessels' diameter and a loss of vasoreactivity [14, 15].

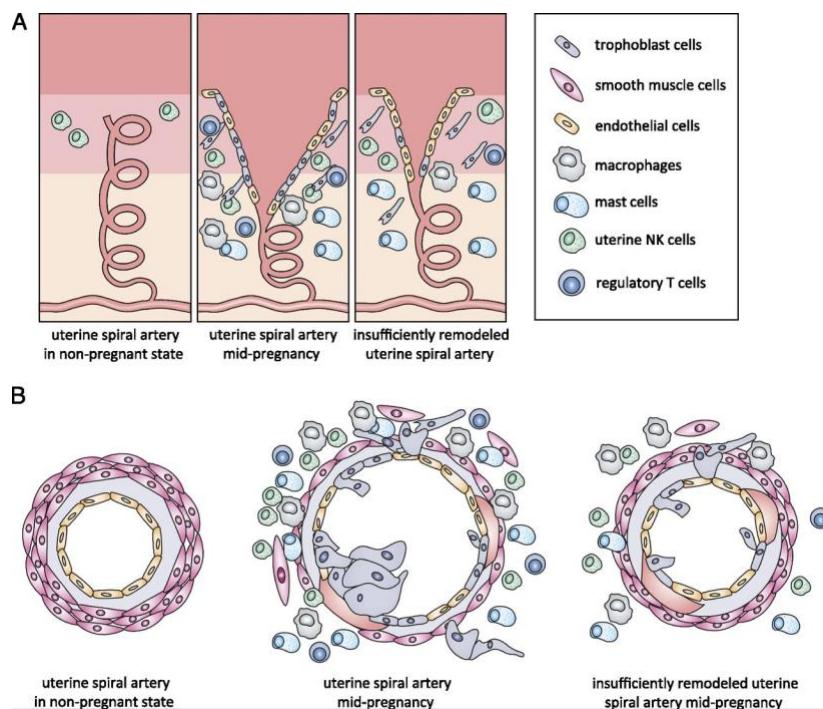
The SpAs lie in the myometrium and extend into the endometrial layer, supplying blood to the outer functional layer, or functional endometrium, of the uterus (**Figure 3**). During pregnancy, this outer functional layer is the layer that transforms into the decidua. In a nonpregnant state, spiral arteries are nonbranching end arteries with a corkscrew shape of about 50-100 $\mu$ m in diameter that penetrate the inner myometrium and the endometrium. Moreover, uteroplacental blood flow in the nonpregnant state is approximately 45mL/min but can increase to about 750mL/min when a pregnant person

reaches term. SpA remodeling is essential to accommodate this immense increase in uteroplacental blood flow during pregnancy. Arterial remodeling is not completed until weeks into gestation, therefore before the formation of a uteroplacental circulation the uterine glands provide necessary nutrients for the fetus. It is not until around the ninth week of gestation that the SpAs are able to supply maternal blood to the intervillous space [16].

The remodeling of the SpAs can be categorized into 5 stages, according to Pijnenborg et al. [17]. Stage one is characterized by a decidua-associated early vascular remodeling where there is endothelial vacuolization and the swelling of individual smooth muscle cells. The second stage consists of the invasion of interstitial EVT—a subpopulation of EVT—into the decidual stroma and perivascular tissue. This invasion causes further disintegration of the vascular smooth muscle and weakens the arterial elastic lamina. In stage three, endovascular EVT—the second subpopulation of EVT—enter the vessel walls via the spiral artery lumen. “The physiological change,” or stage four, commences the physical and physiologic modifications that are necessary for the uterine SpAs to become larger capacitance vessels. Now, trophoblasts can incorporate themselves into the vessel walls causing the formation and spread of a fibrinoid layer that replaces the vascular smooth muscle and elastic lamina. The fifth, and final, stage involves maternal vascular repair with subintimal thickening and reendothelialization [17].

Successful remodeling of the SpAs leads to successful placentation. The human placenta is hemochorial, the most invasive type of placenta, in which the maternal

immune system is in intimate contact with the fetal tissue (trophoblast) in both the placental bed and fetal placenta. In a hemochorial placenta the placental and maternal tissue come in contact in two ways: (1) the outer surface of the placenta is embedded in



**Figure 4: Leukocytic Environment Around the Uterine Spiral Arteries.** The diagram shows the current understanding of the leukocytic environment around the uterine spiral arteries in the non-pregnant and pregnant states. **(A)** Immune cells play vital roles in the remodeling of the SpAs, and if their levels are insufficient during pregnancy the SpAs will not modify to adjust for the increase in uteroplacental blood flow. **(B)** A rich uterine microenvironment is conducive to stimulating trophoblast invasion to facilitate proper SpA remodeling. Taken from (Schumacher et al., 2018) [106].

maternal decidua and (2) maternal blood bathes trophoblasts that are within the placenta in the placental villous tree. The outer surface is the space where placental trophoblasts and uterine leukocytes come into contact, and where an immune response could take place (**Figure 4**). Bathing fetal trophoblasts in maternal blood allows placental antigens

and other factors to be released directly into maternal blood to regulate any maternal immune response [18, 19].

The placenta and/or decidua also release angiogenic factors, growth factors, and other proteins or hormones that can permit trophoblast migration or endothelial-like tube formation. Some of the most significant factors include vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), both pro-angiogenic factors, and two anti-angiogenic proteins, soluble endoglin (sEng) and soluble fms-like tyrosine kinase (sFlt-1) [8]. There is also the release of insulin like growth factor (IGF) and human chorionic gonadotropin hormone (hCG) [15]. Other hormones that contribute to the placental environment and gestation include progesterone and estrogen. hCG production and release is stimulated by the implantation of the blastocyst and helps stimulate fetal organ growth and differentiation and prevent premature uterine contraction. Progesterone plays a role in early pregnancy by inducing the differentiation of stromal cells into decidual cells, which helps reshape the cervix to adapt to fetal implantation. Lastly, estrogen helps induce placental neovascularization [20].

## THE PLACENTAL MICROBIOME AND MATERNAL IMMUNE SYSTEM

These placental factors all help comprise the placental microbiome, a unique microenvironment that undergoes various adaptations and alterations with the means of sustaining a healthy pregnancy. Evidence from the past several years suggests that the feto-placental unit has a bacterial presence, which differs from the old hypothesis that posited that the maternal-fetal unit is germ free. During pregnancy, the physiological changes occurring in the mother's body could be labeled as "immune modulation," which necessitates a balance between immune suppression and strict immunity, both of which are essential in gestation [21]. As stated in Aagaard et al. (2014) [22], the "establishment and maintenance of placental integrity and function are crucial to fetal growth, development, and survival." Aagaard and colleagues analyzed placenta specimens collected from a population-based cohort in order to further study the unique placental microbiome niche. They were able to taxonomically classify the placenta microbiome via whole-genome shotgun (WGS), a method used to sequence multicellular genomes. It was found that the placental microbiome is characterized by a higher abundance of Proteobacteria and the unique presence of Tenericutes, with *E.coli* being the most prevalent single species. Overall, the microbial density in the placenta is low but it maintains a metabolically rich microbiome that is largely composed of nonpathogenic commensal phyla. Apart from the commensal bacteria, an abundant presence of leukocytes at the maternal-fetal border help carry out crucial functions in the aim of sustaining gestation.

All the previously mentioned factors and hormones are a part of the extensive chemokine network needed to control leukocyte recruitment and manage immunomodulation in the expectant mother. In early pregnancy, the chemokine network aids in coordinating leukocyte recruitment to generate the maternal-fetal interface and acts as an additional checkpoint for tissue homeostasis maintenance. Chemokines can mediate the generation of the maternal-fetal interface through the interactions between decidual stromal cells, trophoblasts, and the selective recruitment of maternal and fetal leukocytes. The chemokines can recruit immune cells in inflammation or homeostatic conditions, depending on the synthesis and release of different types of chemokines. Chemokines also contribute to three significant processes needed to sustain embryo implantation: angiogenesis, wound healing, and embryogenesis. For instance, trophoblasts secrete cytokines that regulate the function, differentiation, and recruitment of decidual immune cells. The interaction between CXCL12, a chemokine secreted by first trimester (T1) trophoblasts, and its receptor CXCR4, found on decidual stromal cells, contribute to uterine natural killer (NK) cell recruitment, placentation, implantation, and embryogenesis [23].

Furthermore, proper implantation, placentation, and spiral artery remodeling are all vital processes that need to occur in order to sustain a successful pregnancy with minimal risk for developing a pathology of pregnancy, barring other comorbidities. All these processes are heavily influenced by the maternal immune response that is triggered by the physiological events that precede a possible pregnancy. Immediately after the blastocyst implants into the uterine wall the number of immune cells begin to increase at

the implantation site. Uterine immune cells are essential for the proper regulation of trophoblast invasion and SpA remodeling, thus allowing for proper placentation. The infiltration of these leukocytes also plays a role in protecting the integrity of the uterus [18]. The most pertinent uterine leukocytes involved in ensuring and sustaining a pregnancy are the uterine natural killer (uNK) cells, uterine macrophages, and T cells.

### **Uterine Natural Killer Cells**

Uterine natural killer cells (uNKs) are the most abundant leukocytes during T1, comprising approximately 70% of the decidual leukocytes [24]. Other uterine innate lymphoid cells (uILCs) are also present but in lower numbers. NK cells are a part of the lymphocyte lineage and have either cytotoxic or cytokine producing effector functions. Peripheral NK cells are cytotoxic and kill cells under stress and those that lack 'self' major histocompatibility complex (MHC) class I molecules on the surface of their nucleated cells, suggesting that a large presence of these NK cells could cause fetal cell cytolysis [20]. uNKs are cytokine producing NKs that differ from their peripheral NK counterparts in that they are highly granulated and are identified phenotypically as CD3<sup>-</sup>CD56<sup>+</sup>CD16<sup>-</sup> [12, 18, 24]. They facilitate tissue remodeling, trophoblast invasion, and fetoplacental growth, but they can also inflame and destroy tissues to defend against pathogens. It has even been proposed that uNKs are able to develop "memory" after a first pregnancy, where subsequent uNKs differ from other newer uNKs in that they have a higher expression of genes related to NK cell activation, growth factors, and

immunomodulatory proteins. One of the vital factors produced by uNKs includes the pro-angiogenic factor VEGF [12, 16, 24].

uNKs are found near the site of trophoblast invasion and SpA remodeling sites and it is theorized that uNKs play some role in stimulating both events. Humans have three subsets of uNKs: uNK1, uNK2, uNK3. uNK1 is the most abundant, followed by equal quantities of uNK2 and uNK3. uNK1 cells have cytotoxic abilities and respond to HLA class I ligands, a type of MHC-I molecule. However, following stimulation, uNK2 and uNK3 cells can secrete more cytokines and chemokines than uNK1. These factors include granulocyte-macrophage colony-stimulating factor (GM-CSF) and XCL1, which both act upon EVT. Rather than triggering cytotoxicity, interactions between uNKs and EVTs is said to activate the release of soluble factors that promote maternal spiral artery remodeling. uNK cells can also express certain estrogen receptors, specifically for estradiol (E2), which can directly promote uNK migration and secretion of vascular remodeling factors [11]. It has been established that uNKs produce a variety of growth factors, angiogenic factors, and cytokines that also help promote vascular remodeling and trophoblast invasion. Human uNKs have receptors—KIR, killer cell immunoglobulin-like receptors—that can recognize fetal MHC found on the invading fetal trophoblasts. When uNK KIRs are activated via a uNK/EVT interaction, this stimulates the secretion of GM-CSF and CXCL10, two factors that have been shown to increase EVT migration. uNKs can also secrete TGF- $\beta$  and IL-8 which can reduce EVT migration [24].

Decidual leukocytes, including uNKs, are now understood to be influential in SpA remodeling due to the cooperative activity of placenta derived EVTs and

decidual/uterine immune cells. As EVT's contribute to the loss of the ECM and other vascular cells, SpA remodeling is initiated by the infiltration of decidual leukocytes into the arteriole walls before endovascular EVT's reach the site of remodeling, but the trigger for the immune cell infiltration remains unknown. Research conducted by Choudhury et al. [14] supports the hypothesis that EVT's interact indirectly with SpA endothelial cells to facilitate the recruitment of decidual leukocytes into the arterial wall during early stages of remodeling. Choudhury and colleagues examined T1 placental and decidual tissue obtained from participating pregnant women and dissected and processed said tissues for cell and tissue culture. The cultured human uterine microvascular endothelial cells (HUtMvECs) were either treated with an EVT condition medium or left alone. Their study found that CCL14 and CXCL6, two endothelial chemokines, were both upregulated in the HutMvEC cultures treated with EVT conditioned medium. In vivo studies of decidual SpAs have shown that CCL14 and CXCL6 were expressed in endothelial cells in early remodeling SpAs. Both chemokines' expressions were significantly higher in endothelial cells of remodeling arteries compared to surrounding decidual cells. CCL14 and CXCL6 act as chemoattractants for uNKs and immunofluorescence evidence showed that uNKs have receptors for CCL14 and CXCL6. CCL14 also chemoattracts macrophages, as decidual macrophages have receptors of CCL14. Moreover, secreted factors such as IL-6, CXCL8, macrophage migration inhibitory factor (MIF), MIC-1, and CCL2 play a role in the SpA remodeling process. Choudhury and colleagues discovered that the neutralization of IL-6 and CXCL8 in EVT conditioned medium cultures reduced the ability to upregulate the expression of CCL14 and CXCL6 mRNA in HutMvECs,

potentially impairing SpA remodeling and indicating that IL-6 and CXCL8 are two key activating factors.

### **Uterine Macrophages**

The next most abundant uterine immune cell is the uterine macrophage.

Macrophages (M $\phi$ ) are attracted to the decidua because of the presence of seminal fluid [26]. Growth factors released from the placenta induce a shift in differentiation of uterine M $\phi$  from the M1 (inflammatory) phenotype to the M2 (wound-healing) phenotype [20]. M2 uterine macrophages are immunomodulatory, encouraging maternal immune tolerance and resolution of inflammation. A uterine M $\phi$  expresses various M2 markers, including the following: CD206, CD163, and DC-sign. However, they are not typical M2 macrophages as they are not induced by cytokines secreted by T helper type-2 (Th2) cells—a subtype of T cells—but by macrophage colony-stimulating factor (M-CSF) and IL-10 released by trophoblasts, as seen in in vitro studies. Uterine macrophages are present in the placental bed throughout the entirety of gestation, but they are most abundant during T1 and T2. They participate in SpA remodeling and trophoblast invasion, as well as potentially being involved in blastocyst implantation and protecting the developing fetus against intrauterine infection. Uterine M $\phi$ s produce and secrete factors that are associated with angiogenesis and tissue remodeling. They are typically found near the spiral arteries that exhibit disruption and disorganization in their vascular smooth muscle and endothelial cells. This suggests that they may help prepare the arteries for remodeling. Macrophages also serve as the main antigen presenting cell (APC) at the

maternal-fetal interface during early gestation. The uterine M $\phi$  can engulf apoptotic trophoblast cells to prevent the release of any pro-inflammatory factors [12, 18, 105].

### T Cells

The third most prevalent immune cell at the maternal-fetal interface is the T cell. In early pregnancy, only approximately 10% of the leukocytes at the maternal-fetal interface are T cells [18], but they play a vital role in recognizing and tolerating the invading fetus. The subset of T cells that are most important in immune regulation and maintaining a successful pregnancy are the T regulatory, or Treg, cells. Maternal Tregs have high expressions of CD25, cytotoxic T-lymphocyte antigen 4, programmed cell death 1, and transcription factor Foxp3 (CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>). Tregs are already present in the uterine draining lymph nodes, yet numbers increase during early gestation, which some theorize is due to a rise in the levels of estrogen and progesterone [27]. Data has shown that Treg proliferation is influenced by the presence of seminal fluid as the antigens in sperm can induce the expansion of Treg cells. Studies done on mice populations—who have hemochorial placentas, much like humans—have shown that the factor TGF- $\beta$ , an immunosuppressive factor found in seminal fluid, is seen to drive the proliferation of Tregs and induces tolerance to paternal antigens [26, 27]. The same study determined that the excision of the male mouse seminal vesicles lessened the maternofetal tolerance, suggesting seminal plasma could be important in the induction of tolerance. Some findings suggest that when maternal Tregs recognize paternal alloantigens they become activated and lead to the upregulation of the expression of the receptor CCR5—found on both uNKs and the uterine M $\phi$ s—and the factor CCL3 in the

pregnant uterus. This is said to induce the accumulation of paternal antigen-specific Treg cells in mice [28].

Maternal Tregs contribute to the maintenance of tolerance during pregnancy by suppressing maternal alloreactive immune responses and are also seen to be vital in allowing for proper implantation of the blastocyst into the uterine wall.

CD4<sup>+</sup>CD25<sup>HI</sup>FOXP3<sup>+</sup> Tregs are found in high levels in the decidual tissue and are said to have the ability to suppress fetus-specific and nonspecific responses. There are three subpopulations of Tregs that participate in maintaining pregnancy: CD25<sup>HI</sup>FOXP3<sup>+</sup>, PD1<sup>HI</sup>, and TIGIT<sup>+</sup>. EVT<sup>s</sup> and uterine macrophages favor Treg differentiation into either the CD25<sup>HI</sup>FOXP3<sup>+</sup> or PD1<sup>HI</sup> subpopulation. Both subtypes help modulate cytokine production by T effector cells, thus mediating any potential maternal immune response [29]. Decidual Tregs develop in a way to ensure that they can help prevent destructive effector T cells from responding to fetal antigens, as well as help regulate other decidual immune cells by promoting an anti-inflammatory environment. In a mouse model it was shown that Treg deprivation caused the reduction of maternal vascular adaptation in pregnancy. Other mouse model studies have shown that pregnancy can imprint Tregs with a protective memory to fetal antigens, which can allow for a rapid accumulation of maternal Tregs during any subsequent pregnancy [16].

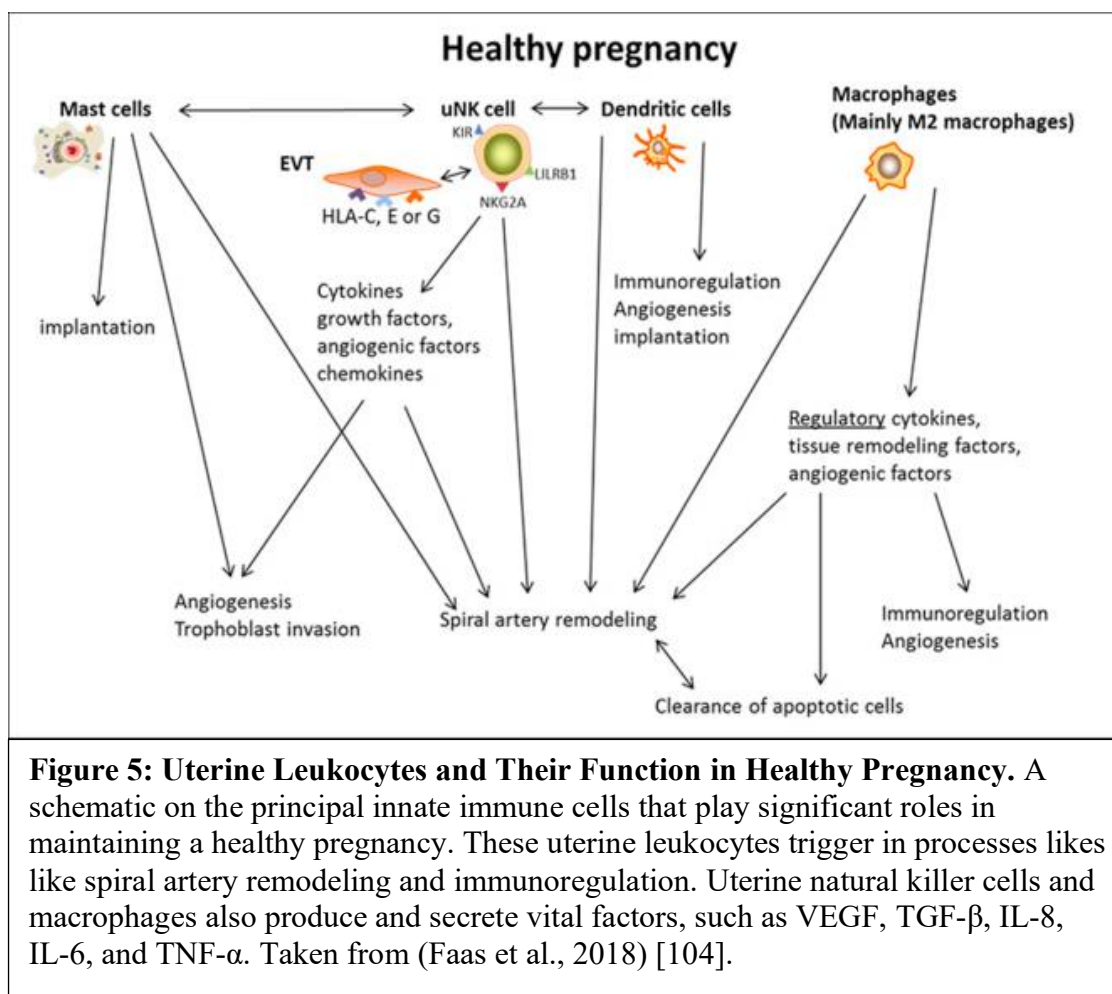
T cell interactions with EVT<sup>s</sup> and uterine macrophages, along with other factors/proteins and the possibility of Treg “memory,” contribute to the priming of the maternal Treg cell population. Studies done with mice have shown that antigen presentation is exclusively mediated by maternal APC<sup>s</sup>, such as uterine macrophages or

other dendritic cells (DCs), and that direct recognition of paternal MHC by maternal T cells does not happen much during pregnancy. T cell recognition is mediated almost entirely by resident DCs presenting paternal MHC antigens or placenta-specific tissue antigens [19]. Placental EVT's only express HLA-C, a key molecule that can elicit an immune response by uNKs and T cells. These HLA-C molecules interact with KIRs expressed on maternal uNK cells. The HLA-C molecules are from both maternal and paternal lineages, so normal placentation requires allorecognition, or ability to distinguish 'self' from 'other,' by maternal KIRs of paternal HLA-C [1, 16]. Recent studies have shown that elevated T cell levels in pregnancy is associated with mismatched maternal and fetal/paternal HLA-C and could cause T cell activation that could lead to various pregnancy complications [19, 27, 29].

### **Other Decidual Leukocytes**

uNKs, macrophages, and T cells are the most abundant of the leukocytes present at the maternal-fetal interface, other uILCs are also present and play roles in sustaining a pregnancy. Neutrophils in the uterine environment function as effector cells. Studies have found that decidual neutrophils (dNs) numbers rise during T2 and under the influence of decidual CXC-motiv-chemokine (CXCL8) can adopt a phenotype similar to other decidual immune cells. A 2014 study showed that dNs display angiogenic properties due to having higher levels of angiogenesis-related proteins (VEGF, ARG-1, CCL2) [30]. Uterine dendritic cells (uDCs), alongside macrophages, serve to present antigens to T cells. uDCs are in cluster-like structures along the uterus and are important for tissue

remodeling and angiogenesis as depleting uDC numbers disrupts uterine integrity and can inhibit implantation [26].



### The Maternal-Fetal Interface

All the previously mentioned factors, chemokines, and leukocytes work in tandem to support and preserve the maternal-fetal interface. Current and emerging data is moving away from the traditional “host graft” model for the maternal-fetal interface that postulates that the presence of immune cells at the maternal-fetal interface is due to an

immune response against the semi-allogeneic fetus. It was believed that the fetal-placental unit, which expresses paternal antigens, is in constant antagonism with the maternal immune system. The relationship between the developing fetus and the maternal immune system was viewed through the same lens as organ transport immunology and is similar to graft-host interactions. It was thought that the failure of maternal immunosuppression was the cause of pregnancy complications like miscarriage and preterm birth [19, 20]. Now, data suggests that the immune response is vital to the reception, implantation, and development of the blastocyst. **Figure 5** provides a brief summary of the principal innate uterine leukocytes and some of their functions. Instead, immunosuppression during pregnancy is not ideal because the deletion of immune cells and the inhibition of specific signaling pathways can lead to pregnancy loss. Normal, healthy post implantation decidua should be rich in immune infiltrates. Current evidence proposes that immune cells are recruited to promote successful pregnancy/implantations and not in response to presence of 'foreign' or 'non-self' fetus [10].

Implantation, placentation, and the maternal immune system/response are all complex and critical processes that need to occur to support a healthy, full-term pregnancy. Preeclampsia, one of the various complications that could threaten the life of the mother and developing fetus, does not have a known etiology, yet current research focuses on how an abnormal maternal immune response affects the numerous physiological adaptations and alterations that sustain a pregnancy. Although PE can affect any pregnant person, those who have comorbidities or any other extenuating factors that could increase one's propensity of developing any complication during

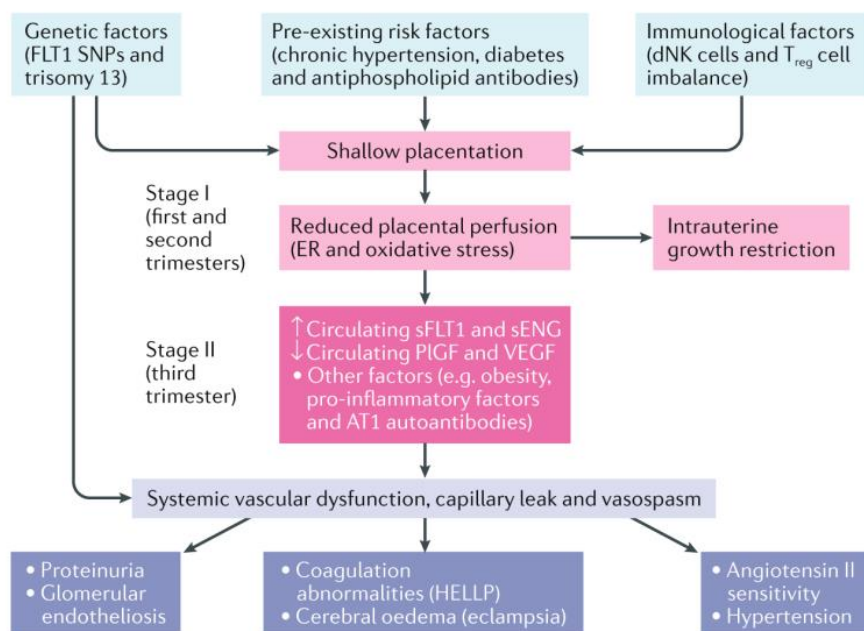
gestation are the most likely to be affected by this disease. One of these factors includes conceiving via ART, such as utilizing IVF. Scholars and physicians are now concentrating on how an aberrant immune response can lead to the pathophysiological manifestation of PE regarding potential atypical implantation and/or placentation.

## THE PATHOPHYSIOLOGY OF PE

Preeclampsia continues to have an unknown etiology, though the pathophysiologies that present in patients with PE are being researched with increasing frequency as the disease affects approximately 5% of pregnancies [1]. According to the National Hospital Discharge Survey, hypertensive disorders of pregnancy—PE, eclampsia, gestational hypertension, and chronic hypertension—were detected in about 5.9% of approximately 39 million US births that were monitored over a 10-year period. Specifically, it was found that people with PE (or eclampsia) were at a 3-25 fold increased risk of developing other obstetric complications during their pregnancy [31] and PE/eclampsia is the likely cause of approximately 50,000 maternal deaths worldwide per year [32]. African American women were found to have an increased risk of PE and other severe pregnancy complications when compared to American Indian, Asian, Hispanic, Pacific Islander, and white women [33]. As noted above, another group of women that has an increased propensity for developing PE include those undergoing assisted reproductive technology treatments. Although different women will experience a different progression of the disease, many will endure some of the same pathophysiological effects and complications that can affect both the mother and the fetus.

Some of the probable pathophysiologies that lead to PE include spiral artery remodeling, deficient placental oxygenation, redox and immune intolerance at maternal-fetal interface, and an imbalance of angiogenic and antiangiogenic factors. There is also an extensive list of potential risk factors and comorbidities that increase the likelihood of

PE, some of which consist of genetic and metabolic conditions like obesity, pregestational diabetes, and a familial or personal history of PE (**Figure 6**) [4]. Normally, the treatment for PE is the delivery of the fetus and placenta [1, 10, 34], although there have been instances when PE develops *de novo* during the postpartum period after the recent delivery of the placenta [4]. The frequency with which PE presents in pregnancies worldwide, along with the severe complications it can bring to groups of women, such as African American women and those undergoing fertility treatments, makes PE a public health concern that needs further research and review. The following section will highlight some of the leading theories on the abnormal physiological events that could lead to the development and advancement of PE and its symptoms.



**Figure 6: The Pathogenesis of Preeclampsia.** The schematic outlines some of the probable etiologies for preeclampsia and how they lead to the classical manifestations of the disease. Taken from (Rana et al., 2019) [4].

### **The Two-Stage Model**

PE has two phases, the placental stage and a stage commonly referred to as the ‘maternal syndrome.’ The first two-stage model was proposed by Redman in 1991 [35]. Redman characterized PE as primarily a “trophoblastic disease,” though the endothelial, renal, and hemodynamic systems are also involved. He contended that the disordered tissue was the placental trophoblast because the presence of a fetus was not needed, nor was the uterus. For instance, people with molar pregnancies—a complication distinguished by an abnormal growth of trophoblast cells in which a noncancerous tumor forms in the uterus due to a nonviable pregnancy [36]—and people with abdominal pregnancies can develop PE. In 1993, Redman and Roberts, further refined the two-stage model by highlighting the importance of endothelial dysfunction during the placental stage of the disease and how it links stage one to the systemic maternal disease in stage two. They postulated that the maternal endothelium is the primary target of the placental-derived factors released in stage one, leading to maternal symptoms like hypertension and proteinuria [37].

Refinements to the two-stage model were consistently made throughout the late 1990s and are still being explored in current research. The most updated model differs from the classical model in that it considers more than one etiology for stage one. Now, the placental stage is described as the physiological abnormalities and aberrant processes that occur in the evolution of the disease. Stage one consists of abnormal placentation and trophoblast invasion, as well as changes in the leukocytic and humoral environment of the maternal-fetal interface. The maternal syndrome stage represents the widespread

symptoms and effects on the mother—and, in turn, the fetus—that emerge because of the events that did, or did not, occur during the placental stage. We see an imbalance in angiogenic factors, increases in free radical production, and endothelial lesions in various organ systems, such as the hepatic or renal systems [1, 3, 4]. The following will discuss some of the possible pathophysiologies that may occur during stage one and how a new emphasis on the role of the maternal immune system, and maternal leukocyte and cytokine imbalances, in PE research seems the most relevant concern in many PE patients, including ART pregnancies, which are at a higher risk for PE.

### **Abnormal Placentation and Endothelial Dysfunction**

One of the most prevalent theories on the potential etiology of PE focuses on the abnormal placentation and vasculature seen in many, if not all, patients diagnosed with PE. PE is a pathology that requires the presence of a placenta. Placentation requires that trophoblasts successfully invade the decidua to promote the development of the maternal and fetal vasculature, as well as to anchor the placenta into the decidua. When placentation is not completed correctly, it can cause obstetric complications that can have adverse effects on both the mother and neonate. Shallow trophoblast invasion has been purported to be a major factor in the development of PE in cases of early-onset PE, which comprise about 5-20% of all PE cases [42]. Although shallow trophoblast invasion does not seem common in PE patients, its effect can lead to other placental and vascular complications that are customarily seen in patients with PE. Trophoblasts are vital in exposing the maternal immune system to paternal antigens, thus contributing to the

process of instilling an immune tolerance in the mother. Trophoblasts also help shape the uterine microenvironment, especially with regards to the maternal leukocytes that play critical roles in initiating and sustaining a successful pregnancy. Trophoblasts also act as immune regulators as they actively respond to the microenvironment and express cell-surface receptors that can recognize specific molecules, like DAMPs and PAMPs [10].

Armant et al. [44] performed a case study in which they aimed to examine how epidermal growth factor (EGF) signaling helps regulate trophoblast differentiation, and how an alteration to the EGF pathway can lead to perinatal diseases/complications. Previous studies have posited that trophoblast survival and their invasive capacity is linked to intracellular signaling by peptides related to EGF. Evidence from *in vitro* cultures has shown that EGF peptides can act as survival factors that protect against apoptosis. It was shown that trophoblast motility and invasiveness is stimulated by EGF, transforming growth factor- $\alpha$  (TGFA) and heparin-binding EGF-like growth factor (HBEGF). HBEGF protects T1 cytotrophoblast cells from apoptosis when under low oxygen concentrations or oxidative stress due to ischemia-reperfusion injury. HBEGF levels are greatly reduced in persons with PE [45, 46]. Armant and colleagues analyzed six EGF family peptides and a truncated EGF receptor splice variant in the placentas of women affected with PE, those who had preterm labor or small for gestational age fetuses, and women who had normal pregnancies. Their analysis showed that HBEGF was mostly found in cytotrophoblast cells of chorionic villi and EVT's, as well as a down regulation of HBEGF, TGFA, and EGF in people with PE when compared to the other comparison groups. These three peptides can alter the integrin expression associated with

trophoblasts' increased motility and invasiveness. They hypothesized that the reduced expression of these three EGF peptides could be due to a decrease in trophoblast cells due to apoptosis or failed invasion. The other 3 peptides analyzed (BTC, AREG, and EREG) were not consistently downregulated in persons with PE. Overall, HBEGF was most significantly downregulated and could be a cause for PE, especially if other factors of the EGF pathway were also reduced and contribute to the suppression of signaling [44].

Placental apoptosis is also seen to be exaggerated in preeclamptic placentas. Apoptosis is a series of energy-dependent events in which one of the goals is to remove unwanted cell material while avoiding an immune response and damage to surrounding tissues. The amount of apoptosis in the placental villi changes throughout the course of gestation, with the least amount of apoptosis occurring in T1 as the rate steadily increases throughout pregnancy. Villous trophoblasts from placentas of pregnancies complicated with PE demonstrate increased susceptibility to apoptosis. In PE there may be a reduction in trophoblast cells within the SpAs, which is seen to be associated with increased apoptosis and reduced luminal size. Poor trophoblast invasion and improper SpA remodeling is suggested to lead to the formation of a high-pressure placental blood supply, causing damage to the developing placental villous tree and altering the placental structure. The damage can lead to placental hypoxia and reduced blood flow, leading to obstetric complications like PE [47].

To analyze the role that apoptosis plays in the development of PE, Travaglini et al. [48] aimed to evaluate the immunohistochemical expressions of a certain antiapoptotic protein, Bcl-2 (B-cell lymphoma/leukemia), along with other angiogenic factors—VEGF,

PlGF, and hepatocyte growth factor (HGF)—that may play a role in initiating the progression of PE. Bcl-2 is a special anti-apoptotic protein that has been shown to prevent apoptosis by blocking cytochrome C release from the mitochondria, and its levels may be depleted in patients with PE. VEGF, PlGF, and HGF are all involved in the remodeling of the maternal vasculature. Their study showed that there was an alteration in the expression of apoptotic, angiogenic, and epithelial factors involved in the placental dysfunctions underlying PE, specifically early-onset PE. PE is often accompanied by hypoxia and reperfusion of the placenta, which can potentially induce apoptosis in trophoblastic cells. In these cases, we also see a decreased expression of Bcl-2. Furthermore, endothelial dysfunction caused by suppression of VEGF signaling has also been postulated to be a major cause of PE. VEGF and PlGF are angiogenic factors that promote pseudovasculogenesis, or the transformation of cytotrophoblast cells from an epithelial to an endothelial phenotype. In cases of PE, increased levels of sFlt-1 led to suppression of VEGF and PlGF signaling, which can potentially explain aberrant vascular remodeling in PE.

Many of the pathological findings associated with the placental syndrome (Stage 1) of PE relate to the aberrant processes that affect the maternal vasculature. In preeclampsia changes to the spiral arteries is restricted to the decidua, whereas in normal pregnancies these changes extend into the myometrium. SpAs that only invade the decidua tend to be narrower and undilated, thus resulting in uterine hypoperfusion [1]. Certain HIFs (hypoxia inducing factors) were also expressed at high levels in proliferative trophoblasts and in preeclamptic placentas [4]. Placental ischemia and

hypoperfusion cause abnormal blood flow, leading to ischemia-reperfusion injury and placental oxidative stress [43].

Spiral artery remodeling relies greatly on trophoblast invasion. Interstitial trophoblast invasion occurs before endovascular invasion. However, in PE interstitial trophoblast invasion is normal but endovascular invasion is shallower [16]. Systemic vascular dysfunction involves an imbalance in the production of constrictor and dilator factors in endothelial cells, reduced endothelium-dependent dilation, and oxidative stress. Circulating factors of placental origin and their interaction with the maternal endothelium are also said to play a role in inducing vascular dysfunction. People with PE present increased total vascular resistance and arterial blood pressure that remains throughout gestation until delivery. Normal vascular adaptations and alterations that occur during pregnancy, like the reduction of peripheral vascular resistance, do not take place. An imbalance in vasoactive factors that either induce vascular smooth muscle relaxation or contraction may explain the hypertensive phenotype in PE [34]. Arteries isolated from persons with PE showed reduced endothelium-derived relaxation, which could partly be attributed to the reduced bioavailability of nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>) [49, 50], which both trigger vascular smooth muscle relaxation.

Impaired placental perfusion leads to adverse placental function and the release of inflammatory placental factors. Human clinical studies have shown that an imbalance in levels of anti-angiogenic and pro-angiogenic proteins and other factors can serve as biomarkers that can be predictive for early detection of PE [51]. Research on angiogenic factors' imbalanced levels in PE discovered that increased sEng and sFlt-1, anti-

angiogenic factors, trap circulating VEGF and PlGF, pro-angiogenic factors [52], reducing their bioavailability, thus helping induce endothelial dysfunction and abnormal placentation. Additionally, mice model studies have been performed to examine the hypothesis that the placental ischemia seen in PE is induced by abnormal uterine artery remodeling causes an increase in the expression and maternal serum levels of sFlt-1. A special mice model was developed to study this theory. Researchers saw manifestations of PE—i.e., maternal hypertension, FGR, and/or maternal vascular dysfunction—in mice that had elevated levels of sFlt-1 and/or endoglin (Eng) [53]. These manifestations were then able to be prevented with delivery of VEGF<sub>121</sub> [54, 55]. Mice with reduced levels of PlGF also presented with PE-like symptoms, primarily maternal endothelial dysfunction [56].

Apart from helping induce poor placental perfusion and placental ischemia, poor SpA invasion can cause oxidative stress. Preeclamptic placentas have increased levels of reactive oxygen species (ROS) compared to antioxidants [4], promoting a state of oxidative stress, vascular inflammation, and vasoconstriction [34]. The imbalance between pro-oxidant and antioxidant mechanisms that could be due to defective spiral artery remodeling is thought to lead to repetitive ischemia-reperfusion injuries, which also increases the production of ROS [1]. Oxidative stress also promotes the elevation of the anti-angiogenic factor sFlt-1 [57]. The heme oxygenase (HO) pathway, an important mediator of oxidative stress, is impaired in cases of PE [1, 4]. Isoforms of HO have been shown to play a role in the vascular function of mother and fetus, as well as placental development and function [58].

Vascular dysfunction during gestation in people with PE may suggest elevated risk of cardiovascular disease (CVD) or acute atherosclerosis in future. Atherosclerosis, sclerotic narrowing of arteries and arterioles, fibrin deposition, and infarcts can all be caused by hypoperfusion and ischemia of the placenta. Acute atherosclerosis is characterized by the presence of CD68<sup>+</sup> subendothelial lipid-filled foam cells—products of inflammatory stress—and fibrinoid necrosis, and it usually occurs at the tips of inadequately remodeled spiral arteries. There are three proposed mechanisms for acute atherosclerosis: (1) decidual inflammation; (2) immunologic mechanisms due to the tips of the affected SpAs being colocalized with cells from a semi-allogeneic fetus and instigating an immune response; and (3) altered hemodynamics, or laminar flow, caused by incomplete SpA remodeling, causing endothelial stress and stimulating the production of foam cells. Acute atherosclerosis is even proposed to be both a consequence of placental dysfunction *and* a risk factor for placental dysfunction [16]. Other potential long term CVD effects in the mother caused by endothelial dysfunction in PE include hypertension, peripheral arterial disease, coronary artery disease, cerebrovascular disease, and congestive heart failure [4, 34].

### **“Immune Maladaptation”**

Normal pregnancy is often described as a mitigated systemic inflammatory response with specific metabolic changes. In cases of PE, this systemic inflammation and metabolic changes become exaggerated and atypical. Proposed pathways in the pathogenesis of preeclampsia consists of theories involving the excessive innate immune response to abnormal maternal physiology, such as a pre-existing endothelial

dysfunction, that may lead to inflammation, angiogenic factor imbalance, endothelial and/or placental dysfunction, and PE. Other pathways propose that an enhanced or abnormal innate immunity before and/or during gestation may lead to complications [60]. Contemporary ideas on the immune system's role in the pathophysiology of PE posits that an abnormal maternal immune response against the placenta may represent one of the first pathological steps of the disease, followed by the systemic inflammation of the maternal endothelium [61].

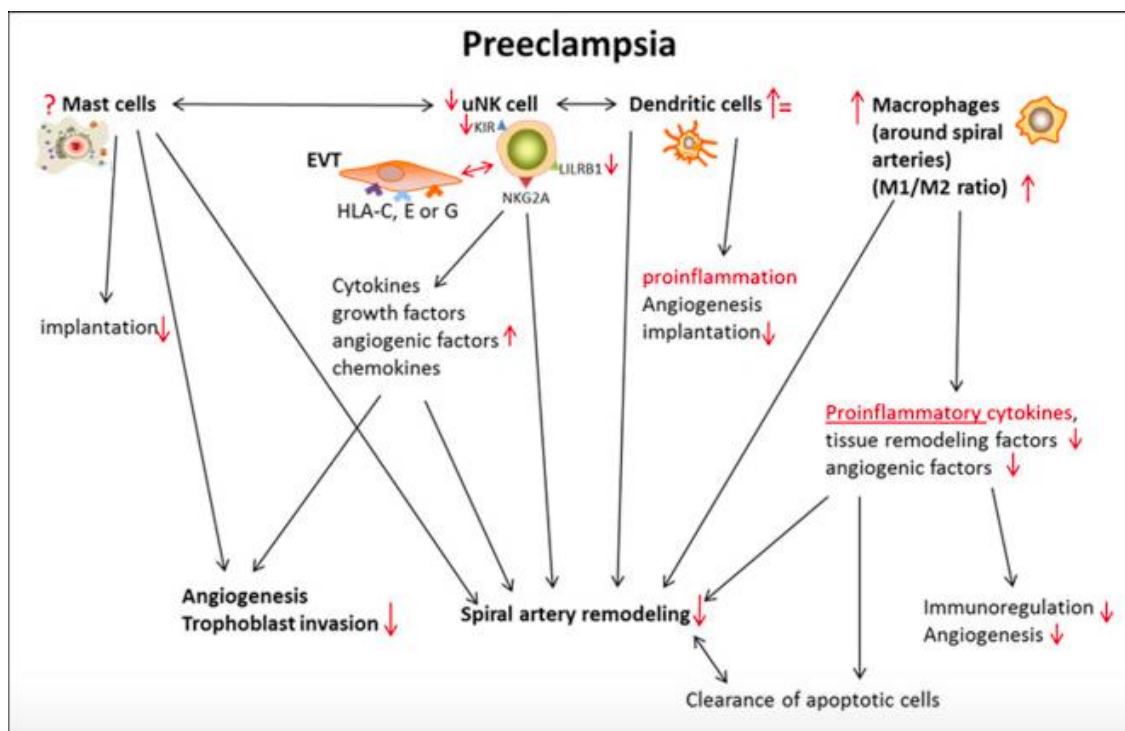
With the rising interest in the roles of the various leukocytes that compose a portion of the uterine microenvironment, the maternal immune system has been hypothesized to be involved in the progression of PE. One of the leading theories concerning the immunity component of PE is that it is caused by some sort of "immune maladaptation," as it is referred to by Kenny and Kell (2017) [59]. Because the fetus is semi-allogeneic to the mother due to half of the fetal antigens being of paternal origin, there is an 'immunological conundrum' that is present during gestation. Therefore, the mother needs to have immune tolerance towards the developing fetus as we now recognize that there are constant interactions between material of maternal and placental/fetal origin throughout pregnancy. Furthermore, the significance of the uterine leukocytes and maternal immune response during pregnancy, and their involvement in processes like implantation and placentation, make research into the immune system's function in PE a growing field.

The altered immunomodulation of the maternal immune system is seen as the first possible pathological insult of PE. Normal pregnancy is understood to be a Th2

(suppressor T helper cell) immunological state in which the Th2 lymphocytic response increases in proportion to the Th1 (pro-inflammatory T helper cell) numbers to favor immune tolerance and lower cytotoxic activity to prevent fetal rejection. Consequently, preeclampsia is described as a Th1/Th2 imbalance where the normal shift towards Th2 preference is altered to a higher circulating Th1:Th2 ratio [59,61]. The abnormal gestational ratio will now resemble the Th1 levels of non-pregnant women, with a cytokine profile favoring the production of pro-inflammatory cytokines like IFN $\gamma$ , TNF- $\alpha$ , and IL-6. Along with the enhanced activation of M1 (inflammatory type) macrophages, PE also presents with a decrease in circulating Tregs and uterine Tregs and an increase in the levels of pro-inflammatory Th17 cells. The Treg/Th17 ratio imbalance bolsters the increase in the secretion of inflammatory cytokines [11, 61].

Other cytokines exacerbate the systemic inflammation and cytotoxic uterine environment seen in preeclampsia. PE patients express an increase in circulating levels of TNF- $\alpha$  and IL-6 while IL-10 and IL-4 levels decrease, which stimulates inflammation and contributes to processes that support endothelial dysfunction [62]. These imbalances are mainly caused by the altered leukocytic levels in the preeclamptic placenta. Uterine NK cells and M $\phi$ s produce most of the factors mentioned, with Th2 cells producing IL-4 (**Figure 7**). TNF- $\alpha$  reduces the mRNA of NO synthase, lessens acetylcholine-induced vasodilation, and increases the production of ET-1, a potent vasoconstrictor, from endothelial cells. Measured circulating levels of TNF- $\alpha$ , IL-6, and ET-1 show that they are increased 2-3 fold in persons with preeclampsia when compared to normotensive women. Moreover, high levels of TNF- $\alpha$  and IL-6 can induce trophoblast apoptosis.

Decreased levels of IL-10, an anti-inflammatory cytokine secreted by Tregs and involved in regulating the immune response, aids in bolstering the levels of TNF- $\alpha$  and IL-6 as IL-10 is implicated in reducing the levels of Th1 cells [61].



**Figure 7: Uterine Leukocytes and Their Function in a Preeclamptic Pregnancy.**

The schematic shows the alteration in the leukocytic levels and function of a preeclamptic pregnancy. In PE there is a decrease in the levels of activated uNK cells, which are responsible for producing and releasing cytokines and other factors that contribute to a healthy pregnancy. There is also a shift in the M $\phi$  activation profile and ration, favoring the more cytotoxic M1 phenotype, which contributes to the development of PE. Taken from (Faas et al., 2018) [104].

Other contested theories involving the maternal immune response and PE include the potential adverse effects of endogenous molecules expelled by damaged or injured tissues (i.e. DAMPs), dormant microbes, and/or foreign pathogens may have during gestation, like excessive inflammation and oxidative stress [8, 10]. Research has shown

that the mitochondria play a role in regulating and maintaining the inflammatory response. The theory posits that the immune system may become activated through the release of mitochondrial DAMPs that result from mitochondrial dysfunction and/or mitochondrial-derived ROS (mROS), prompting inflammation. Two recognized mitochondrial DAMPs are mitochondrial DNA (mtDNA) and N-formyl peptides, both of which have elements that are conserved from the bacterial origins of mitochondria. DAMPs act as “danger signals” that bind to specific receptors on innate APCs, activating a signaling cascade that promotes the production of cytokines meant to eradicate the stressor. It has been shown that maternal serum levels of free mtDNA, as well as placental-derived mtDNA, is increased in PE patients. mtDNA can bind onto a particular receptor on neutrophils, leukocytes belonging to the innate immune system, activate them, and trigger the formation and release of neutrophil extracellular traps (NETs). An increased NET formation is seen in cases of PE. This leads to the upregulation of pro-inflammatory transcription factors, and thus an increase in the production and release of pro-inflammatory cytokines [63-65]. Additionally, the mitochondria are major producers of ROS. mROS formation and release is increased in PE due to a distortion in mitochondrial function and the exaggerated activity of the placental electron transport chain, which was seen to correspond with the elevated soluble levels of anti-angiogenic factors recorded in PE patients when compared to normotensive patients. An excess in the formation of mROS will result in oxidative damage and inflammation [66-68].

The potential introduction of active infectious agents can also be a challenge to pregnancies and lead to abnormal responses and adaptations that could cause the

development of preeclampsia. The human vaginal microbiota is key in the defense against infection. The vaginal microbiome changes significantly during gestation, with an overall decrease in bacterial diversity, increased stability, and the enrichment of the *Lactobacillus* species of lactic acid producing bacteria. All these changes are consistent with the decrease in the vaginal pH and an increase in the vaginal secretion of metabolites that help inhibit bacterial and viral infections in the urogenital tract. The vaginal microbiome changes accordingly depending on the gestational stage [21, 72]. A study on the vaginal microbiome has shown that certain vaginal communities in early gestation were correlated with an increased risk of having a preterm birth. These bacterial communities include a low amount of *Lactobacillus* sp. and an increased abundance of *Ureaplasma* and *Gardnerella* species [73].

The activation of dormant microbes and the possible role of infectious agents serve as other emerging theories on the etiology of PE. Bacterial infections can disrupt immunological milieu created at maternal-fetal interface and lead to obstetric complications. The infectious agents can provoke an immune response that may lead to excessive inflammation and potential injury or death to the cellular components of maternal-fetal unit. It has been established that immune cells promote fetal acceptance, but they can also create an overwhelming response to an infection that can prompt fetal rejection [10]. Kell and Kenny (2016) [8] argue that dormant microbes may become reactivated and begin to shed inflammagens. A dormant microbe is one that is in a low metabolic state where the organism does not appear to be viable, but it is not dead. A dormant microbe can become reactivated and exit the state of dormancy. For instance, the

reactivation of dormant infectious agent *Porphyromonas gingivalis* causes LPS shedding [71]. Lipopolysaccharide (LPS) has been shown to inhibit EVT invasion and stimulate the production of pro-inflammatory cytokines IL-6 and IL-8 [69]. As previously explored, the effects of LPS could be related to abnormal placentation and excessive inflammation, both commonly observed in PE patients. LPS has also been used in rodent models to trigger symptoms similar to those seen in humans/primates with PE [70].

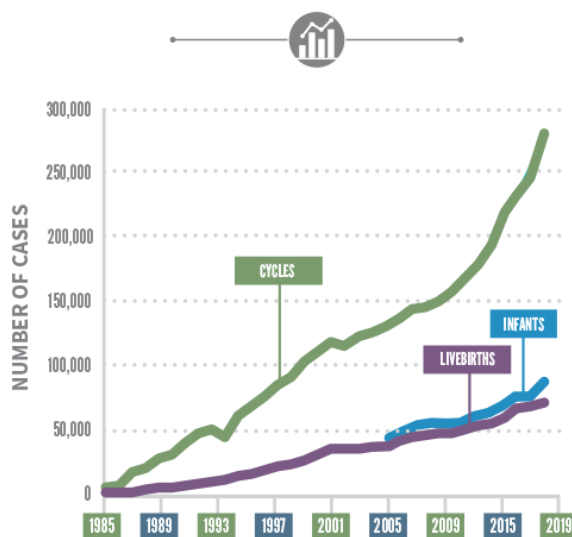
Lastly, the paternal effect cannot be overlooked when considering a potential maternal immune response that could lead to abnormal maternal physiological responses. Some researchers contend that a change in partner can minimally increase the risk of developing preeclampsia. PE has been commonly related to first pregnancies with a new partner because of the shorter exposure period of paternal antigens to the maternal immune system. Trophoblasts must express paternal alloantigens that must be recognized by the mother's immune system, and HLA-C are the only classical HLA I antigen expressed on EVTs. Therefore, a maternal and fetal/paternal HLA-C mismatch could induce an immune response that would result in an obstetric complication, like PE, especially—according to those who ascribe to this theory—in couples who have had shorter, unprotected cohabitation periods. Conception after the use of a barrier method contraceptive has also been said to increase a person's susceptibility to preeclampsia. HLA-C mismatch appears viable impetus the activation of the maternal immune response. However, the cohabitation theory has been highly contested as many studies have also found that there is no significant difference in rate of PE between long-term

partners conceiving for the first time and shorter-term partners conceiving for the first time [59, 74-76].

## ASSISTED REPRODUCTIVE TECHNOLOGIES: A RISK FACTOR FOR PE?

The first baby conceived via *in vitro* fertilization technology—the first ART procedure to be developed and widely used—was born in 1978. Since then, with approximately 5 million people having been conceived via ART by 2013, nearly 0.1% of the global population, numbers of ART conceptions and births continue to steadily increase as the technology and society continue to evolve throughout the years [79]. Current estimates propose that IVF and other ART methods are responsible for about 7

### Number of Cycles, Deliveries and Babies



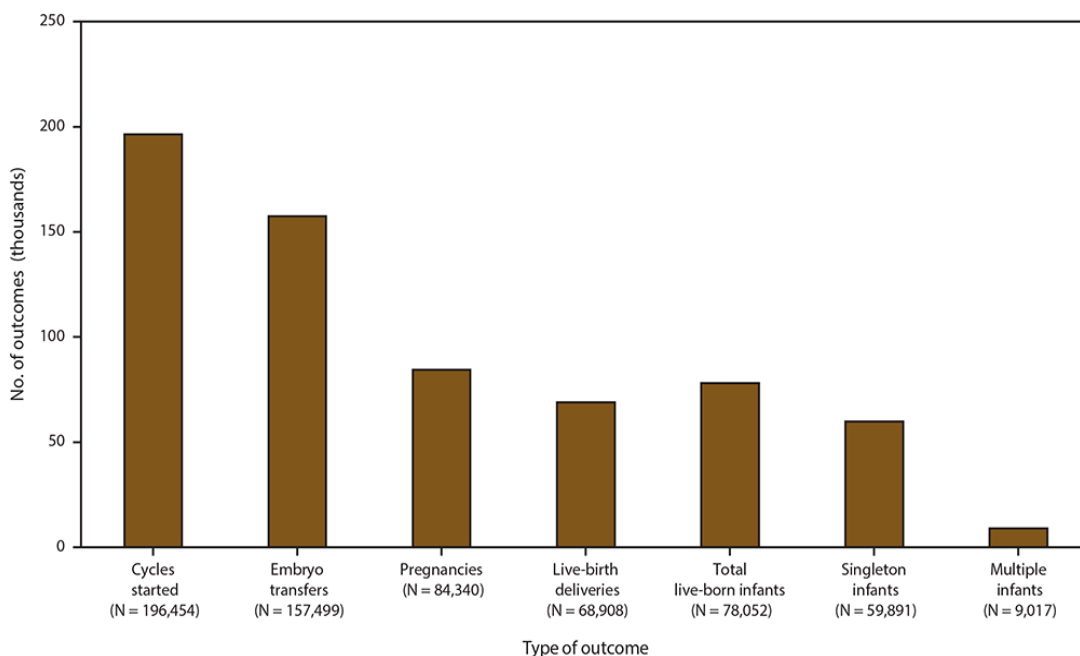
**Figure 8: Number of Cycles, Deliveries and Babies.** A physical representation of the rise in ART cycles, or rounds, which have significantly increased in the past 35 years. The number of live births and infants associated with successful ART cycles. Taken from the Society for Assisted Reproductive Technology [77].

million births worldwide as of 2021 [82]. **Figure 8**, taken from the Society for Assisted Reproductive Technology (SART), shows the growing rate at which ART techniques are being implemented in the United States. In the SART infographic the number of ‘cycles’

of ART has increased significantly since the beginning of the widespread use of this technology in the late 1970s. ART procedures include IVF-ET (IVF embryo transfers), IVF with OD, IVF with donor sperm, ICSI, FET, and intrauterine insemination (IUI). IVF, or a combination of another ART protocol in conjunction with IVF, comprise the vast majority of ART cycles performed. A ‘cycle’ indicates a singular instance in which a patient undergoes a transvaginal oocyte retrieval, a patient receives a donor oocyte, the patient undergoes egg freezes/fertility preservation treatments, or a donor donates oocytes. This is followed by the insemination of the oocyte—with partner or donor sperm—and an embryo transfer. A patient who is having their own oocyte retrieved during their ART cycle may have one or more oocyte retrieved during the procedure [80]. As of 2019, ~275,000 cycles of ART are performed per year in the US. Alongside the increase in ART cycles performed per year, there has also been an increase in the number of livebirths seen per year as a result of the yearly cycles. The yearly growth in livebirths credited to ART signifies the advancements made throughout the years in protocols and monitoring, as more babies can be successfully brought to term.

More specific data from the 2017 United States “Assisted Reproductive Technology Surveillance” (**Figure 9**) [78] survey the various outcomes of ART procedures to monitor the state-by-state implementation of ART, as well as the birth outcomes in any given year compared to non-ART births. In the year 2017, there were over 196,000 ART cycles started, with about 68,000 cases culminating in a live birth which contributed 1.9% of all infants born in the United States. A majority of these pregnancies and births were singleton, but ART-conceived multiples are still common

and appear to be more often associated with an obstetric or fetal complication. As a suggestion on public health action, the researchers who compete the yearly “Assisted Reproductive Technology Surveillance” report recommend that increasing the use of single-embryo transfers (SET) could help reduce any potential health consequence for either the mother or infant. While the interest and use of ART continues to grow, it is imperative to investigate the possible challenges that could arise as a product of how these assisted conceptions come to fruition.



**Figure 9: Number of outcomes of ART procedures, by outcome type.** \*A total of 284,403 of ART cycles were reported to the CDC in 2017. This figure excludes 87,931 cases in which egg or embryo banking was performed and 18 experimental cycles used to evaluate a new treatment procedure. Taken from (Sunderam, 2020) [78].

Ischemic placental disease, or IPD—which includes PE, placental abruption, and small for gestational age fetus—contributes to more than half of the medically induced

deliveries before 35 weeks gestation, as well as half of all preterm births. IPD is also more common in ART pregnancies than in spontaneous conceptions, with ART pregnancies more likely to be complicated by a disease like preeclampsia [5]. The risk of obstetric complications further increases with multiple pregnancies, so there has been a drop in multiple embryo transfers and a rise in SET, which is common in FET, an increasingly popular ART protocol due to the rise in the use of cryopreserved embryos. However, various studies have shown that FET has a higher risk of PE when compared to other ART procedures, such as fresh embryo transfer or standard IVF with autologous oocytes [81-83]. Researchers now question whether the increased prevalence for PE could be due to a particular ART method's protocol and how said protocols can affect the maternal physiological adaptations and alterations needed for pregnancy. It is also seen that general risk factors for PE—maternal age, change of partner, long birth intervals, smoking, and/or other medical comorbidities—are frequently seen in patients looking into ART [82-86]. Considering preeclampsia's unknown but complex etiology, why and how the rate of PE can be greater in ART pregnancies is a pressing discussion as more people look toward the use of the technology in the future.

### **Standard IVF and Combined ART Procedures**

For this review, 'standard' IVF is classified as an ART cycle that utilizes IVF with an autologous oocyte and partner sperm for insemination. A general IVF cycle will last roughly 4 weeks and, in brief, include oocyte retrieval or donation, insemination, and oocyte transfer into the uterus [80]. A study performed by Gui et al. (2020) [6] followed a

population of 114,484 pregnant women in Nanjing, China, between the ages of 18 and 35 who delivered between the years 2013-2018, of whom 96% conceived spontaneously and 4% using IVF. They identified a total of 1339 cases of PE, with a higher incidence of PE in IVF pregnancies, 6.1%, versus spontaneous pregnancies, 1.0%. The IVF procedures that were utilized were standard IVF, IVF-ICSI (used to assist the fertilization process if the male partner's sperm count is abnormal or if the partner has unfit sperm [80]), and some cases of fresh or frozen embryo transfer. It was noted that the mothers undergoing IVF were older compared to the mothers who conceived spontaneously. They calculated that, for this study population, the risk of developing PE was more than 2-fold higher following IVF. They highlighted that confounding risk factors included primiparity, the patient's weight, and multiple pregnancies.

Gui and colleagues emphasize that IVF techniques could lead to disruptions in the endometrial microenvironment as a result of the conceptus being transferred into the uterine cavity via the cervix. This is proposed to cause an alteration in hormone levels, which could disturb the development of the maternal-fetal interface and lead to placental dysfunction. Aligning their theory with one proposed by Thomopoulos et al. [86], they argued that because the formation of the chorion is initiated in vitro in IVF pregnancies, there must be some difference in the constitution of the placenta that may predispose a patient to certain obstetric complications, such as defective placentation, placental vascular lesions, and insufficient uteroplacental blood flow. These complications increase the risk of presenting an IVF-mediated hypertension phenotype.

A 2021 assessment by Ganer Herman et al. [87] went a step further and studied the possible obstetric outcomes of IVF in a cohort of women where each woman acted as her own control. They identified a population of women who had previously conceived without the help of ART then underwent ART for a subsequent pregnancy, excluding the use of OD. It was noted that the maternal age at delivery at the time of the IVF pregnancy was higher than when the spontaneous conception occurred. They determined that PE occurred in 1.3% of the IVF pregnancies compared to 1.1% of the spontaneous pregnancies, deriving that there was not a significant difference in obstetric complications in IVF versus natural conceptions in the same mother. However, it was mostly seen that mothers with other factors that could increase the risk of developing PE had a higher rate of PE than mothers who developed PE just because they underwent ART. Overall, reviews and analyses following the rate of preeclampsia in ART pregnancies demonstrates an increased risk for PE if the patient is employing an ART method to conceive.

### **Donors and ART**

Even though PE rates are likely increased with the use of any ART method, the PE rates are more significantly increased in ART cycles that call for the use of donor oocytes and/or donor sperm. The risk for developing preeclampsia in an oocyte recipient increases 4-fold in comparison to spontaneous conceptions. The maternal immunologic response is posited to be the most probable etiology in oocyte recipients who developed PE. Some studies have even shown that there is a decreased rate in PE when a donated

oocyte came from a relative. On average, patients in the oocyte donation process tend to be older, and it is recognized that the PE risk is higher in patients over 40. To a large extent, studies and meta-analyses comparing pregnancies using OD to spontaneous conceptions, or conceptions resulting from IVF without OD, consistently find an increased risk of PE in the OR group, even when controlling for maternal age. Patients who underwent OD still has a higher risk of PE even when compared to women of similar age who underwent IVF without OD [7].

Oocyte donation has been implemented in ART protocols almost for as long as ART has been utilized. Early studies on the clinical applications of oocyte donation conducted in the mid-1990s [88] showed that those undergoing oocyte donation have a higher risk of developing obstetric and neonatal complications, with the risk of PE in OD pregnancies being calculated at approximately 32.7%. Although recent PE risk probabilities in OD gestations are not as high, meta-analyses and studies on published research surrounding OD in ART protocols demonstrated that the PE risk was higher in oocyte donation pregnancies than conceptions via other ART methods and natural conception, both singleton and multiple gestations [82, 89]. Recent studies are now grappling with the same question: do IVF pregnancies using OD have higher complication rates than IVF-ICSI pregnancies using autologous oocytes?

Tarlatzi and colleagues [90] aimed to properly analyze this risk while ensuring that they control for confounding factors, like age, parity, and multiplicity. Their study was performed with a cohort of women with singleton pregnancies that received a donated oocyte and gave birth to a baby after 22 weeks gestation, with the control group

being women with singleton IVF-ICSI induced pregnancies using autologous oocytes. Women with multiple pregnancies, mothers with genetic diseases that could lead to pregnancy complications, and pregnancies using cryopreserved embryos were excluded from the study. There was a total of 244 women—144 in each group—with the mean age of the participants being 35.64 years old. They discovered that in this cohort, the rate of PE was found to be higher (12.5%) in conceptions using OD versus those using autologous oocytes (5.6%).

The higher incidence of PE in OD ART protocols is proposed to be due to the fetus being entirely allogeneic; the fetus does not have a genetic connection to the maternal host. This causes a potential immunologic challenge to the maternal immune system [61]. Recently, a study [91] found that an increased number of fetal-maternal HLA class II mismatches are associated with PE development in OD gestations. It was suggested that there is an activation in the maternal T cell response. Possibly, fetal cells expressing paternal and donor antigens may migrate into the maternal circulation and lead to a maternal immune response that is more pro-inflammatory and unregulated. OD gestations require a different type of immunoregulation when compared to spontaneous conceptions. OD—and ART pregnancies in general—show a higher number of activated T cells, and increased Treg numbers, in the maternal host. OD gestations also showed decreased levels of IL-10 and IL-6, both anti-inflammatory cytokines [92]. It was also found that because of the allogeneic fetus, monocytes (precursors to macrophages) may be activated differently or more strongly, as the M $\phi$  presence must be even more essential due to the immune burden. Studies reviewed by Tian et al. [93] showed there was

aberrant M $\phi$  function in OD pregnancies when compared to IVF or spontaneous pregnancies. There are different M $\phi$  subtypes that contribute to various physiological processes needed to maintain a pregnancy. The ratio of these subtypes is altered to maintain an OD pregnancy.

An emerging theory involving the role, or lack thereof, of the corpus luteum (CL) in certain ART pregnancies, like those involving OD, are being investigated to determine if the CL may play a role in helping create the environment that induces the development of PE or hypertension of pregnancy. It is hypothesized that IVF pregnancies have a higher PE rate because certain ART protocols effect the hormones in uterine environment. The corpus luteum is a major source of hormones during gestation, especially during T1. OD pregnancy protocols are programmed IVF cycles that lead to no formation of the CL, whereas unassisted pregnancies have the formation of one CL. Because of the lack of the CL, OD ART protocols require the administration of estradiol and progesterone during the cycle, but other vasoactive factors otherwise released by the CL are not exogenously administered. For instance, relaxin, a vasoactive hormone secreted by the CL, helps in maternal cardiovascular adaptation during gestation. Relaxin is not commonly administered in OD protocols and its lack can be attributed to less compliant vasculature, thus increasing PE risk in pregnant people without a CL. The impact of not having a CL is most significant in T1, mostly corresponding with no/less circulating CL-derived factors. Studies have shown that persons with no CL had higher rates of PE and severe PE than women who formed one, or multiple, CL(s) in their gestations [83].

Similarly, the use of sperm donors in certain ART pregnancies is posited to be associated with a heightened propensity for preeclampsia. Donor sperm would be used in IVF and IUI treatments for various reasons, like if a partner has a low sperm count or defective sperm, if a single individual wants to conceive on their own, or in cases of same sex relationships in which neither individual can provide sperm [75]. It has been hypothesized that prolonged exposure to a partner's sperm may reduce the risk of developing PE as the maternal immune system would have established some tolerance to the 'foreign' material and induced some sort of protective effect, potentially reducing the risk of PE. Thus, a lack of sperm exposure and short-term sexual cohabitation is associated with higher risk of PE and pregnancy-induced hypertension because it is argued that prolonged exposure to partner's sperm can alter the maternal immune response to their partner's antigens [94].

A meta-analysis [75] aimed at evaluating if insemination using donor sperm can increase the risk of PE when compared to pregnancies conceived with a partner's sperm completed in 2014 evaluated a total of 10,898 women for gestational hypertension and preeclampsia. Of the cohort of women, 8556 women conceived with partner sperm and 2342 with donor sperm. All seven of the studies included found higher rates of PE in women who conceived with donor sperm, 212 out of 2342 cases, than those who conceived with partner sperm, 461 out of 8556 cases. Four of the studies found similar rates of gestational hypertension in both groups. It could be concluded that donor sperm notably increases the risk of PE compared with pregnancies conceived with partner sperm.

A study published in 2019 by Bartal et al. [94] proposed to explore the hypothesis that conceptions using sperm donation (SD), or gamete donation in general, show an increased propensity for preeclampsia. They gathered a group of women who underwent ART cycles using donor sperm and a partner oocyte or a donor oocyte and have a singleton pregnancy. Conceptions achieved using a partner oocyte and donor sperm were not seen to be associated with a higher risk of PE and other obstetric or neonatal complications. Women who had IVF-SD + OD conceptions were at a greater risk of developing PE when compared to women underwent IVF-SD or IUI-SD. Therefore, they found that double gamete donation has the greatest propensity for PE. They noted that women who had a double gamete donation were on average older and had higher rates of having previous medical conditions. Nonetheless, their findings point to the idea that previous exposure to sperm before insemination does not reduce the risk of complications.

It was once believed that long periods of cohabitation with the same partner, maybe along with the use of non-barrier method contraceptive, could decrease a person's susceptibility for PE. This theory based itself on the idea that a women's immune system needs to become slowly exposed and tolerant of their partner's antigens [59, 74, 75, 94]. The theory behind this concept has pivoted to resemble that of OD gestations. Instead of heavily relying on the 'change of partner' or 'cohabitation period' aspect of the theory, ideas are now depending on the possibility of an aberrant immunologic response as the principal source for the pathophysiology of the disease.

It is necessary to recognize that seminal plasma, the non-sperm component of seminal fluid, is most essential to priming. It has been seen that the excision of seminal vesicle glands lessened the tolerance-inducing effect of seminal fluid. On the other hand, having a vasectomy, which removes sperm from seminal fluid, does not have a great influence on the priming response. Seminal fluid is a source of TGF- $\beta$  and prostaglandin E (PGE), both factors that act as Treg cell-inducing agents and induce the expansion of paternal antigen-reactive Tregs. Because there are typically a few days between coitus and implantation, if insemination occurs, the seminal fluid has time to prime and induce appropriate Treg differentiation. TGF- $\beta$  and PGE-related prostaglandins, specifically 19OH-PGE<sub>1</sub> and 19OH-PGE<sub>2</sub>, help induce T cells into Tregs that express Foxp3. Seminal fluid was also found to encourage uterine expression of mRNA that encodes for CCL19, a Treg chemokine that regulates T cell retention. Uterine cells were identified as a major cellular origin of CCL19, which allows for T cell accumulation before implantation [95].

### **Cryopreservation and FET**

Frozen/fresh embryo transfer—notably the use of frozen, or cryopreserved embryos—has risen in demand as more individuals choose to forgo having children ‘early’ and hope to leave the possibility of facilitating conceiving at ages in which either partner is less reproductively fit. FET has been associated with better outcomes because it does not require the use of controlled ovarian stimulation (COS), which can damage the endometrium and uterine environment, and its cycles are conducted in a slightly modified “natural” uterine environment [96, 97, 100]. Despite its rise in popularity, frozen embryo

transfer, FET, carries one of the highest risks of developing preeclampsia in comparison to most other ART methods, even fresh embryo transfer [83, 81]. Studies and meta-analyses comparing FET to fresh SET-IVF or other ART methods that do not use cryopreserved embryos have largely deduced that patients who underwent FET had higher cases of PE and other obstetric complications [82, 98, 99].

Much like the hypotheses surrounding OD pregnancies high propensity for preeclampsia, the theories on why FET gestations have a higher rate of PE center on the treatment's protocols. Comparable to OD procedures, FET is performed as programmed cycles where estrogen and progesterone are given to prepare the endometrium for implantation. FET cycles suppress ovulation, so the corpus luteum does not form [81, 100]. The absence of a CL results in the lack of production and secretion of not only estradiol and progesterone, but also vasoactive factors like relaxin and VEGF. These vasoactive products are not administered to patients in a standard FET protocol. Von Versen-Höyneck et al. (2019) found that ART methods using programmed cycles (i.e. FET or OD) associated with higher PE rates. Again, the impact of having no CL is most detrimental in T1 of gestation because of the lack of necessary vasoactive products that may lead to vascular dysfunction. However, it was seen that FET protocols that do not significantly modify the natural cycles not associated with increased PE risk [83]. An FET protocol that does not call for the suppression of ovulation typically leads to the formation of one CL, possibly reducing the risk of developing preeclampsia [101].

### **PE and ART: A cacophony of risk factors or a problem with the protocols?**

As the etiology of preeclampsia remains uncertain it is difficult to deny that some sort of alteration to the uterine environment and the typical and necessary maternal immune response. It appears that the increased propensity for PE in ART pregnancies is also connected to pathological reactions that modify the protein/factor, humoral, and leukocyte concentrations and functions. Yet, it remains unclear on whether the increased risk for PE in ART pregnancies is due to the methods/protocols, or if confounding maternal risk factors (advanced age, obesity, change of partner, etc.) play a greater role in increasing the propensity for the disease. Various studies have shown a consensus that use ART can be predictive for hypertensive complications during pregnancy, especially if other confounders are present. The alterations to the uterine environment are of particular concern in ART protocols that call for the transfer and implantation of the conceptus and in procedures that cause an imbalance of vasoactive factors [85, 86].

A cohort study performed a couple of years ago explored the potential factors that could lead to development of PE in women undergoing ART. As formerly mentioned, the prevalence of preeclampsia has been seen to increase with advanced maternal age, change of partner, longer interbirth intervals, obesity, and smoking. Information from the Medical Birth Registry of Norway (MBRN), which contains details on all births in Norway since 1967, was used to compare whether certain confounding factors affect the PE rate in people with spontaneous pregnancies and women who had ART pregnancies. They discovered that people using ART were about 5-9 years older during their first pregnancy, and 3-7 years older in their third pregnancy. If a person had a second and/or

third pregnancy, a higher percentage of women treated by ART had new partners. Also, women who underwent ART had longer interbirth intervals when compared to those who conceived spontaneously. They calculated that, on average, ART pregnancies had mean interbirth intervals of 6.2 years between the first and second conception, and 7.3 years between the second and third conception. Women who conceived spontaneously had interbirth intervals of 3.8 years between the first and second pregnancies and 4.6 years between the second and third pregnancy. Longer birth intervals tend to be seen in cases associated with new partner, may indicate the presence of an infertility problem, and/or are associated with advanced maternal age. For people with more than one pregnancy, the risk of PE increases with a longer birth interval. In this cohort of women who had given birth, the odds of PE were higher in ART gestations, regardless of parity, in comparison to spontaneous pregnancies. Spontaneous conceptions had the following PE rates in, up to, three pregnancies: 5.1%, 2.2%, and 2.1%. Subsequently, those rate in ART conceptions were 6.0%, 3.3%, and 4.4% [84].

There have been many studies and metaanalyses that have found an association between ART and developing PE, however even though this correlation is rampant in the literature some studies have found that the increased risk was not statistically significant. Herman and colleagues work with mothers who have both conceived spontaneously and with the help of ART did not find a statistically significant difference in risk rates between ART and natural conceptions in same mother [87]. Bartal et al. also raise the conundrum that people who used a barrier method contraception with a partner were not seen to have higher risk for obstetric complications once they conceived [94]. The use of

barrier method contraception and short unprotected sexual cohabitations intervals have been seen not to increase the risk for PE [102], further complicating the immunologic theory behind PE and its relation to ART.

## CONCLUSION

As one of the pathologies of pregnancy considered a leading cause of maternal and fetal morbidity and mortality, preeclampsia remains a widely studied but not entirely understood disease with a variety of probable etiologies as outlined in **Figure 6**. Aside from PE's link to increased risk of maternal and fetal morbidity, it is also associated with short and long-term consequences for both mother and baby post-delivery. Potential consequences for the neonate include low birth weight, especially in cases of early-onset PE. There is a heightened risk for preterm birth, which is the world's leading cause of fetal mortality and morbidity. Preterm births relate to higher rates of sepsis, infant respiratory distress syndrome, intraventricular hemorrhage, and neurodevelopmental disabilities in childhood. People who suffered from PE have the potential of developing chronic hypertension, ischemic heart disease, thromboembolism, an increased risk of stroke, or other forms of CVD [4, 34, 103].

With preeclampsia's danger to the health outcomes of mother and fetus/neonate, better comprehension into its pathophysiology and the individuals that are most likely to be affected by the disease has dominated the literature and research into PE. Known complications such as oxidative stress and a pro-inflammatory environment plausibly affect the physiological processes connected with preeclampsia, the most pressing theories and hypotheses center on immunological maladaptations that relate to maintaining these complications. Alterations to the leukocytic milieu, leukocytic function, and the associated protein and humoral imbalance in the uterine environment have been consistently discovered to be involved in the presentation of many PE cases.

Abnormal Treg and Th1/Th2 ratios, favoring the more pro-inflammatory Th1 cells, enhanced activation of inflammatory M1 Mφs, and an increase in the levels of proteins, factors, and products—like TNF-α, IL-6, ROS, and sFlt-1, to name a few—promote an inflamed and cytotoxic uterine environment that could lead to placental and endothelial dysfunction. Uterine immune cells play vital roles in all processes necessary to sustain a pregnancy to term with minimal complications. Environments in which their function becomes impaired raises the risk of a gestational complication or development of a pathology of pregnancy, like preeclampsia. For this reason, events such as ART cycles, and/or because of the contribution of other confounding factors, pose risks to the essential physiological processes needed to ensure a pregnancy.

ART has greatly benefited millions of individuals and families across the world, but as is the case with many medical technological advancements, an increased risk of encountering an obstacle throughout, or because of, the process may be likely. For pathologies of pregnancy, like preeclampsia, research should continue to focus on their increased prevalence and association of with ART. Despite cases of cohort and research studies that show that ART pregnancies do not always show a statistically significant increase in risk for PE, higher rates of hypertensive disorders of pregnancy have been consistently recorded in individuals who conceived via ART. Even between the numerous types of ART procedures, procedures involving donor gametes and those whose protocols modify the uterine environment appear to be associated with the most risk. ART cycles utilizing donor gametes—whether oocyte(s), sperm, or both—present immunological complications, with factors such as fetal-maternal HLA mismatches,

decreased levels of IL-10 and IL-6, both anti-inflammatory cytokines, and a strong M $\phi$  activation. ART protocols with a donor oocyte and those using cryopreserved embryos, FET, may lead to the absence of a CL, which lessens or completely ceases the production and secretion of certain vasoactive factors.

Shifts in social patterns have begun to normalize postponing childbirth and more individuals, and couples, with the need for ART have begun to use its techniques for conception. As ART's popularity and accessibility rises, and its procedures are refined and improved, further inquiry into the common pathologies of pregnancy that may afflict people undergoing ART are necessary to continue to advance the medical technique's efficacy and safety.

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**CURRICULUM VITAE**

