Factors Involved in the Ethiology of Early Onset Preeclampsia and New Possible Treatments

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Abstract

Preeclampsia (PE) is a hypertensive disorder during pregnancy, seen after 20 weeks of gestation, which often occurs with proteinuria. Although PE is the second most common complication in pregnancy, with a prevalence of 5-8% in all pregnant women, the ethiology of PE is still unknown. This thesis focusses on new possible therapies that prevent the adverse effects on mother and child seen in PE. The focus is thereby on the factors involved in the ethiology of PE, including immune cells, placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin.

Risk factors vary widely from ethnicity, to patients suffering from chronic hypertension. PE negatively affects both mother and child. Maternal complications include Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP syndrome) and cerebral haemorrhage, while fetal complications include stillbirth and pre-term birth which can lead to several diseases and associated problems. PE can either have an early onset (< 34 weeks gestation) or a late onset (≥ 34 weeks gestation), from which this thesis focusses on the early onset PE.

Because the ethiology is still unclear, the treatment for PE is limited to early delivery and medication use including aspirin, statins, metformin and esomeprazole. However, these drugs do not inhibit the progression of the disease. Therefore, new effective treatments are needed. The second part of this thesis focusses on the possible therapies, such as toll-like receptors (TLR) antagonists, placenta-derived mesenchymal stem cells (MSCs), PlGF and relaxin to hamper the progression of PE.

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Introduction

Preeclampsia (PE) is a hypertensive disorder that occurs during pregnancy, meaning that the maternal blood pressure is too high (Kattah & Garovic, 2013). PE is often seen after 20 weeks of gestation and is present in 5-8% of all pregnant women worldwide, thereby making it the major cause of maternal, as well as, perinatal morbidity and mortality (Michalczyk et al., 2020). Hypertension in PE often occurs with one of the following conditions: proteinuria, maternal organ dysfunction, or uteroplacental dysfunction (Bokslag et al., 2016). All these symptoms make it dangerous for both mother and child. PE is one of the most common complications in pregnancy, but the ethiology and pathogenesis are still unknown (Wang et al., 2022). Therefore, further research is needed, which will hopefully lead to new targeted therapies, thereby reducing the maternal and perinatal morbidity and mortality. This thesis focusses on the possible therapies that prevent the adverse effects on mother and child seen in preeclampsia. The primary focus will be on the early onset of preeclampsia.

PE can either have an early onset (<34 weeks gestation), or a late onset (≥ 34 weeks gestation). Early onset PE is present in 5-10% of all PE cases, making it less prevalent than late onset PE (Marín et al., 2020). Despite this prevalence, early onset PE has higher rates of maternal and perinatal morbidity and mortality (Bokslag et al., 2016). Early onset PE is associated with a deficiency in the remodelling of the uterine spiral arteries (Aneman et al., 2020). The remodelling of the uterine spiral arteries is needed for maintaining a normal pregnancy, and deficiencies in this remodelling may result in various pregnancy disorders, including preeclampsia (Whitley & Cartwright, 2009). Late onset PE is associated with maternal endothelial dysfunction (Aneman et al., 2020). Endothelial dysfunction is characterized by an impaired vasodilatory capacity, meaning that the blood vessels of the heart constrict, instead of dilate (Opichka et al., 2021). This constriction can lead to hypertension in the mother.
As mentioned before, the exact ethiology of PE is still unknown, but it is believed that PE is associated with the release of soluble factors in the circulation, such as vascular endothelial growth factor (VEGF) and antiangiogenic factors, including soluble fms-like tyrosine kinase 1 (sFlt-1), and soluble endoglin (sEng) (Opichka et al., 2021). sFlt-1 and sEng are both secreted by the placenta (Agarwal & Karumanchi, 2011). In healthy pregnancy, the soluble factors are needed for a proper development of the placenta. However, in PE, the soluble factors act differently than in healthy pregnancy, which makes these factors interesting for new prospects for the treatment of PE.

The placenta is a vital organ with numerous functions including immune functions, endocrine functions and physiological functions. The placenta consists of various features, such as the parenchyma, chorion, amnion and umbilical cord (Herrick & Bordoni, 2023). The placenta consists of fetal tissues, and maternal parts. The fetal tissues form the chorionic sac and the allantois while the maternal part, also called the decidua, consist of three parts: the decidua basalis, the decidua capsularis and the decidua parietalis (Herrick & Bordoni, 2023).
An important feature of the placenta is the chorion and consists of the cytotrophoblasts, syncytiotrophoblasts, and extraembryonic mesoderm. These trophoblasts play an important role in the development of the placenta in healthy pregnancy, but act differently in PE (Reister et al., 1999).

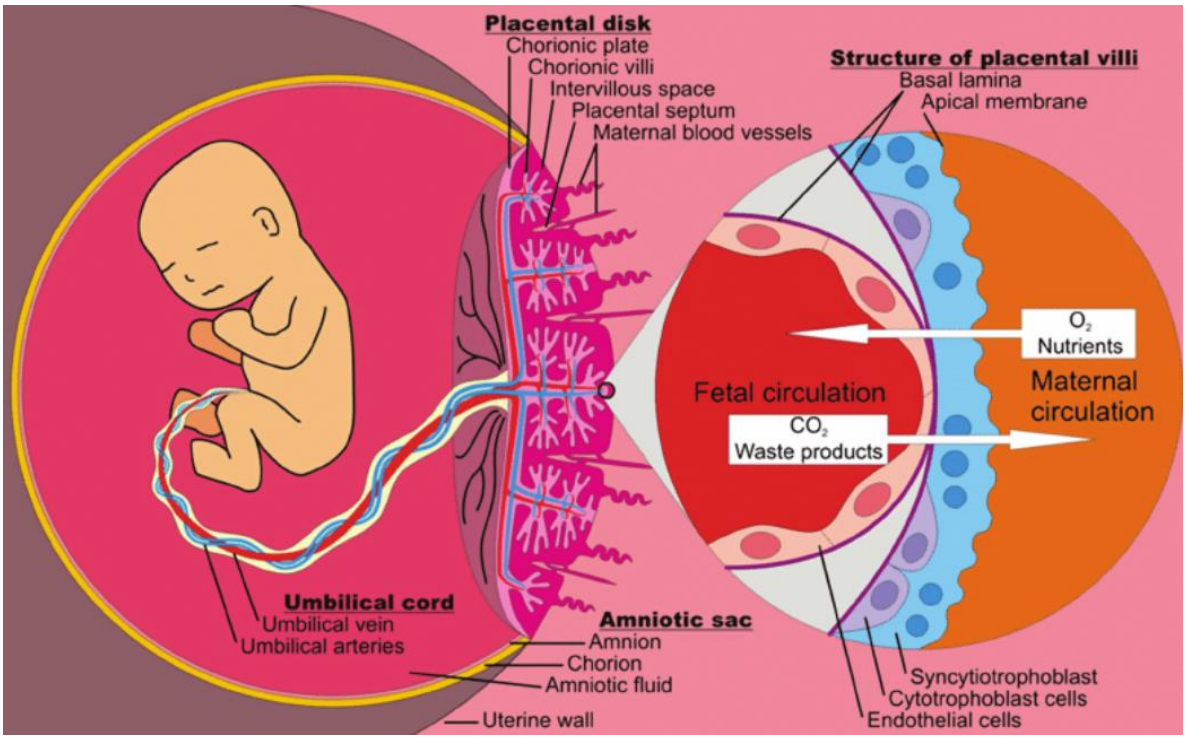

Figure 1. Anatomy of the placenta. The different structures such as the amnion and chorion can be seen. The structures of the different cells involved, including syncytiotrophoblasts, cytotrophoblasts and endothelial cells, are depicted on the right side of the picture (Elad et al., 2014).

There are several risk factors for developing PE, including maternal age, having overweight or obesity, and having a history of diabetes (Yang et al., 2021). A strong risk factor for PE is multiple gestations, and research showed that even ethnicity plays a role. Adding to this, women who have chronic hypertension, autoimmune disorders, kidney disease and history of preeclampsia are also at risk for developing PE (Aneman et al., 2020).

PE has an effect on both mother and child, as PE in the mother can result in Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP syndrome), but also in renal failure (Nirupama et al., 2021). Because of the hypertension in PE, women suffering from PE are more prone to cardiovascular disease. Also, the consequences of severe hypertension during PE can result in maternal cerebral haemorrhage, which is the leading cause of maternal death (Bokslag et al., 2016). Complications of the mother differ from those seen in the child. Complications in the child include stillbirth, with a seven time increased risk in women with PE, compared to women without PE. Fetal growth restriction is often seen in PE, meaning that the child has a 5% lower birth weight compared to a child whose mother did not suffer from PE (Bokslag et al., 2016). Besides stillbirth, preterm birth is also seen in PE. Preterm birth in PE increases the rates of several diseases, such as respiratory distress syndrome, bronchopulmonary dysplasia and neurodevelopmental disability in childhood (Bokslag et al., 2016).
Altogether, PE includes several risk factors, and can have numerous effect on the mother, as well as the fetus. Due to a great variety of the effects, treatment becomes even more needed.

As mentioned before, PE is a hypertensive disorder. The maternal systolic blood pressure in normal pregnancy should be less than 120 mmHg, and the maternal diastolic blood pressure should be less than 80 mmHg. Precautions should be taken into account, especially after 20 weeks gestation, when the maternal systolic blood pressure raises above 140 mmHg, and the diastolic blood pressure raises above 90 mmHg (Kattah & Garovic, 2013), as this might indicate the presence of preeclampsia. Research into preeclampsia was hampered for many years due to the lack of suitable preclinical

models, as the disorder is exclusive to humans. However, several model systems have been introduced that mimic the human phenotype of PE. The vast majority of the models make use of rodents, but sheep, apes, rabbits and guinea pigs have also been proven for a suitable model (Turbeville & Sasser, 2020). These models are needed to understand the mechanisms seen in PE, and test targets of possible treatments.

Despite suitable model systems, there are currently no curative therapies for PE, which necessitates new therapies. Early delivery is being used to decrease the complications seen in PE. Also, there are no drugs available that influence the disease progression (Chappell et al., 2021). However, multiple drugs are being used to hamper the complications seen in PE. These medications include; aspirin, statins, metformin and esomeprazole.

In order to find new possible treatments for PE, this thesis will first discuss the possible ethiology of early onset PE and which factors are involved, followed by the current medications used for PE. This search be done by performing a literature search using the electronic databases of Pubmed Medline, and Smartcat RUG. The focus was on the ethiology of PE and new treatments. The following search terms were used: ''preeclampsia'', ''ethiology'', ''treatments AND preeclampsia'', and ''factors AND preeclampsia''. At last, new therapies will be discussed that will hopefully prevent early onset PE.

Healthy human placentation

Uterine spiral artery remodelling is essential for a successful pregnancy, as it supplies blood into the placenta. Various cells are involved in remodelling, such as immune cells, extravillous trophoblast cells (EVTs), endothelial cells, and vascular smooth muscle cells (VSMC) (Pan et al., 2022). Spiral arteries contain endothelial cells on the inside, and VSMCs on the outside. Uterine spiral artery remodelling involves EVT invasion. In normal pregnancy, the EVT invasion develops deeply into the myometrium, as seen in figure 3, which is the outer layer of the uterus. This invasion stimulates remodelling of the spiral arteries into high flow vessels (Rana et al., 2019). The remodelling phase can be EVT-independent, but also EVT-dependent. Thereby, EVT invasion can either be interstitial via the decidua, or endovascular via the distal ends of the spiral arteries (Whitley & Cartwright, 2010).

The EVT-independent phase, via the decidua, involves endothelial vacuolation, and VSMC swelling. These processes happen prior to EVT invasion. In the EVT-independent phase, decidual leukocytes (DLs), which are mostly macrophages or natural killer cells, infiltrate into the arterial wall, as seen in figure 2B. This infiltration causes disturbances and partial loss of the VSMC layer. This process is followed by swelling of the endothelial cells (Sato et al., 2012). After this, EVT invasion occurs, which results in morphological changes of the VSMCs, thereby migrating away from the vessel wall (Pan et al., 2022). Because of this process, the vessel wall is replaced by intramural EVTs fixed in extracellular matrix (ECM). The remodelled vessels protect the developing placenta, by allowing a gentle flow of high volumes of maternal blood to the intervillous space (Pan et al., 2022).

In the EVT-dependent phase, the EVT differentiate from cytotrophoblasts cells, and thereby obtain expression of non-classical HLA molecules. The cells invade the decidua in the myometrium as far as possible, either through the interstitial route, or the endovascular route (Pan et al., 2022). An overview of these routes can be seen in figure 2C. Invasion via the interstitial route involves breakdown of the VSMC layer, and secretion of fibrinoid material. Due to this, interstitial trophoblasts get fixed with fibrinoid material, and some change into starlike-shaped trophoblasts (Sato et al., 2012). Invasion via the endovascular route involves endovascular trophoblasts moving along the arterial lumen. Thereby, the endovascular trophoblasts replaces the endothelial cells from the inside, and also facilitate the breakdown of the VSMC layer (Sato et al., 2012).

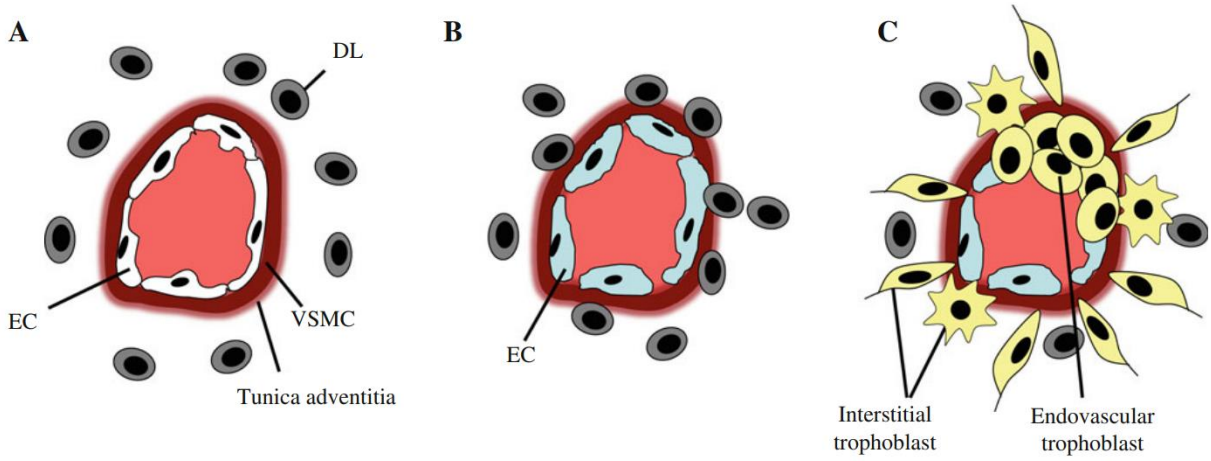

Figure 2. Events during healthy human placentation. (A) Unremodelled artery. Decidual leukocytes (DLs) are present near the artery. (B) EVT-independent remodelling. DLs infiltrate the arterial wall, which causes partial loss of the VSMC layer. Swelling can be observed in the endothelial cells (EC). (C) EVT-dependent remodelling. This process can occur via two routes; interstitial route, and the endovascular route. In the interstitial route, interstitial trophoblasts destroy the VSMC layer and secrete fibrinoid material. This leads to stellate-shaped trophoblasts. In the endovascular route, endovascular trophoblasts try to replace the ECs from the inside, and also facilitate the breakdown of the VSMC layer (Sato et al., 2012).

Placentation in early onset PE

Early onset PE is only present in 5-10% of all PE cases, but its high rates of maternal and perinatal morbidity and mortality makes it relevant for research. Early onset PE is also known as placental preeclampsia, as the placenta is the cause of early onset PE. Early onset PE is characterized by a poor development of the cytotrophoblasts, leading to an impaired villous growth (Marín et al., 2020). In healthy pregnancy, spiral artery remodelling happens in the myometrium, while in PE, this spiral artery remodelling only takes place in the decidua (Faas et al., 2014). This impaired artery remodelling leads to a narrowed vessel, resulting in a decreased blood flow, by which the speed increases. Figure 3 illustrates the differences between spiral artery remodelling in normal pregnancy, and PE. The blood enters at a higher speed which can damage the fetal villi, and it can also damage the placenta. A narrowed artery is prone to atherosis, which is an early stage of atherosclerosis (Kim & Kim, 2015), and decreases placental flow (Rana et al., 2019). Thereby, because of the impaired artery remodelling, oxygen is deprived, resulting in a condition called placental ischemia (Nirupama et al., 2021). This process, on its turn, increases the levels of angiogenic factors, such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng). These angiogenic factors contribute to endothelial dysfunction (Nirupama et al., 2021).

In conclusion, a normal development of spiral arteries is necessary to maintain a healthy pregnancy. In PE, there is impaired remodelling of the spiral arteries as it only takes place in the decidua, instead of in the myometrium. This leads to oxygen deprivation which increases the levels of several angiogenic factors contributing to endothelial dysfunction.

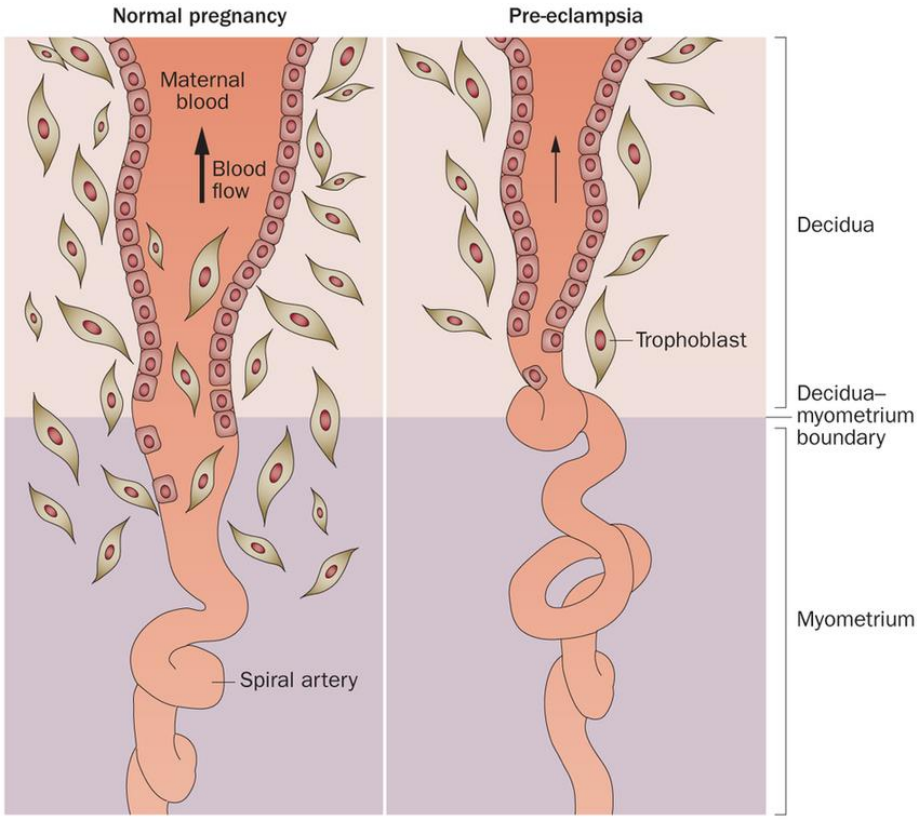

Figure 3. Spiral artery remodelling in normal pregnancy (left) in comparison to spiral artery remodelling in PE (right). In normal pregnancy, trophoblasts infiltrate the myometrium and a wide vessel is present. In PE, the trophoblasts do not infiltrate the myometrium and stay at the level of the decidua. The vessel seen in PE is narrowed, leading to a decreased blood flow compared to normal pregnancy. A decreased blood flow results in blood entering at a higher speed, which can damage the fetal villi and placenta (Chaiworapongsa et al., 2014).

Other factors playing a role in early onset PE

Early onset PE is mainly due to abnormal placentation. This abnormal placentation is the result of placental hypoxia. Hypoxia hampers the placental growth factor (PlGF). PlGF is an important molecule in PE, and belongs to the VEGF-family. PlGF can bind to either VEGF-receptor 1 (VEGFR-1), or sFlt-1. Because PlGF is part of the VEGF-family, is plays a significant role in angiogenesis, which is important for embryonic development and growth (Chau et al., 2017). PlGF is also important for the placenta, because PlGF promotes the development and maturation of the placental vascular system. However, in early onset PE, PlGF is hampered, meaning that the development and maturation of the placental vascular system is abnormal, indicating for instance, abnormal vessel formation (Chau et al., 2017).

The sFlt-1/PlGF ratio is a successful indicator for the prediction of PE (Verlohren et al., 2022). It was shown that when the levels of PlGF were low, the levels of sFlt-1 increased. sFlt-1 is a soluble splice variant of the VEGF-receptor 1. sFlt-1 are made by trophoblasts and are present in the circulation during pregnancy. In healthy pregnancies, sFlt-1 acts as a regulator of the EVT invasion, and thus regulates an appropriate depth of the placenta in the uterine wall (Jena et al., 2020). sFlt-1 is influenced by the oxygen-sensing protein Jumonji domain-containing protein 6 (JMJD6), as JMJD6 influences the splicing of sFlt-1 in endothelial cells. It was observed that the loss of JMJD6 in hypoxic conditions, seen in PE, leads to a greater production of sFlt-1. This greater production of sFlt-1 contributes to PE, because it is responsible for endothelial dysfunction (Palmer et al., 2015).

Although sFlt-1 contributes to the onset of PE, it is unlikely that sFlt-1 on its own controls the onset. It is therefore believed that sEng also contributes, together with sFlt-1, to endothelial dysfunction. sEng is a co-receptor of transforming growth factor β (TGF-β). TGF-β regulates numerous processes, under which vasculogenesis and inflammatory responses (Margioula-Siarkou et al., 2022).
A regulation factor regarding sEng is hypoxia, as the hypoxia-inducible transcription factor 1α (HIF-1α) affects specific sites of the endoglin promotor. This enables the transcription and synthesis of endoglin (Margioula-Siarkou et al., 2022).
In PE, sEng is overexpressed in EVT cells and syncytiotrophoblasts. In healthy pregnancies, there is a controlled increase in sEng. However, due to the hypoxic conditions seen in PE, there is an uncontrollable overexpression of sEng, which leads eventually to endothelial dysfunction. There is also evidence that sEng expression is enhanced by oxidative stress (Margioula-Siarkou et al., 2022).

Oxidative stress occurs when there is an imbalance between pro-oxidant factors, and anti-oxidant factors. In this imbalance, the pro-oxidant factors exceed the anti-oxidant factors, thereby leading to the production of reactive oxygen species (ROS). ROS is essential for the succession and development of a healthy pregnancy. In a healthy pregnancy, antioxidant defences are present to maintain a proper balance between the pro-oxidant factors, and the anti-oxidant factors (Harmon et al., 2016). However, during early onset PE, this proper balance is disrupted. The anti-oxidant defences are lessened, and there is an increase in pro-oxidant factors leading to oxidative stress. This oxidative stress results in inflammation in the vasculature of the placenta, which eventually results in endothelial dysfunction through the release of immune cells (Harmon et al., 2016).

An overview of normal and abnormal angiogenesis, and the balance between the factors can be seen in figure 4.

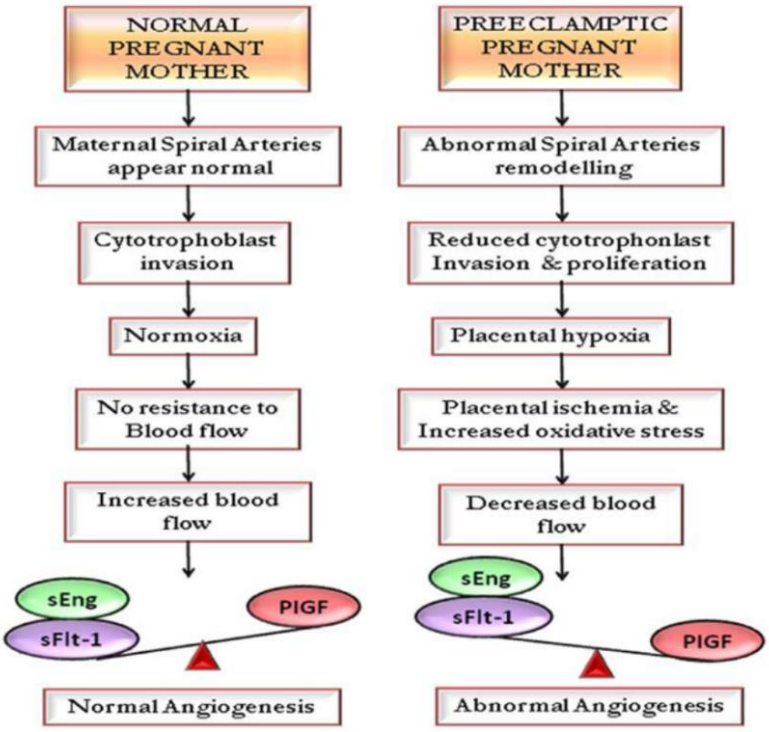

Figure 4. Overview of the processes involved in normal pregnancy, and PE. In normal angiogenesis (left) there is less sEng and sFlt-1, and higher levels of PlGF present. In abnormal angiogenesis, seen in PE, there are higher levels of sEng and sFlt-1 present, and lower levels of PlGF (Nirupama et al., 2021).

Immune cells in healthy human placentation

Although uterine spiral artery remodelling need EVTs, it is believed that decidual immune cells, such as decidual natural killer cells (dNK-cells), decidual macrophages, T-cells and dendritic cells (DCs) also play a role (Pan et al., 2022).

The dNK-cells have a different phenotype than peripheral NK-cells and are only present when remodelling takes place. The dNK-cells are located near the invasion of the EVT, indicating that they might play an important role in modulating the degree of the invasion (Liu et al., 2017). In spiral artery remodelling, the dNK-cells can have a direct interaction using VSMCs, endothelial cells, pericytes and EVTs, or they can have an indirect interaction, which uses factors that are secreted by the dNK-cells. During remodelling, the dNK-cells aggregate around the spiral arteries. This accumulation leads to disturbances of the VSMC layer (Liu et al., 2017). As mentioned before, the disturbances of the VSMC layer is needed for the layer to be replaced by EVTs. These remodelled vessels protect the developing placenta.

Besides dNK-cells, also macrophages are believed to play an important role in human placentation. Macrophages are the second most common leukocytes, and are, in the placenta, closely located to the spiral arteries during trophoblast invasion, and spiral artery remodelling. Due to this location, macrophages are believed to play a role in vascular remodelling. They are believed to induce apoptosis of damaged cells, and remove the apoptotic cell debris. The induction of apoptosis regulates the trophoblast invasion, and the removal of apoptotic cell debris protects adjacent cells from inflammatory reactions (Liu et al., 2017).

Other immune cells that are important for the regulation of EVT invasion, are T-cells. These T-cells include T-helper cells (Th-cells). Figure 5 shows the balance of Th-cells seen in pregnancy during each trimester. The first trimester consists mainly of Th1-cells as this is needed to regulate the EVT invasion, and to stimulate the repair of uterine epithelium and the removal of cellular debris (Graham et al., 2021). In the second trimester, a Th2-cell environment is needed for fetal growth. In the last trimester, mainly Th1-cells are present which are required for parturition (Graham et al., 2021).

All the immune cells above can be studied, but it turned out that decidual dendritic cells (dDCs) are quite difficult to study, as there is not a specific marker. However, DCs are believed to play an important role in the maintenance of fetal tolerance (Liu et al., 2017). Further research is being done regarding the role of DCs in implantation and decidualization. The role of DCs maintaining the uterine vascular cells is still unknown. Also, the idea that sFlt-1, derived from DCs, stabilizes the vasculature, seem to be inconsistent with the thought that DCs regulate angiogenesis through the production of VEGF-A itself (Tagliani & Erlebacher, 2011). In conclusion, the role of DCs in pregnancy is currently still unclear.

In conclusion, immune cells such as dNK-cells, macrophages, Th-cells and dDCs are needed for healthy human placentation. Both dNK-cells and Th-cells are of importance in the regulation of EVT invasion, while macrophages are important for vascular remodelling. dDCs are believed to play a role in maintenance of fetal tolerance, but further research into dDCs in pregnancy is needed.

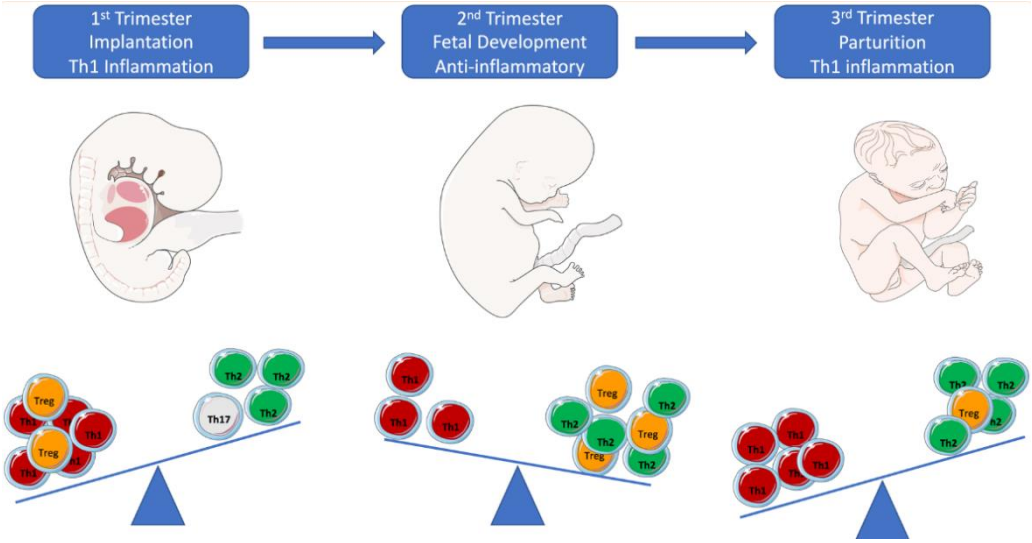

Figure 5. Th-cells balance during each trimester. In the first trimester (implantation), there are mostly Th1-cells needed for the regulation of EVT invasion. The second trimester (fetal development) has a Th2-cell environment which is needed for fetal growth. The last trimester (parturition) consists mainly of Th1-cells again, needed for delivery of the baby (Graham et al., 2021).

Immune cells in early onset PE

The immune cells (dNK-cells, macrophages, Th-cells and DCs) needed for the maintenance of a healthy pregnancy, act differently in PE.

As mentioned above, dNK-cells are important for spiral artery remodelling by producing cytokines and chemokines. It has been shown in women suffering from PE, that they have an impaired dNK-cell function (Zhang & Wei, 2021). This dysfunction of the dNK-cells leads to an impaired spiral artery remodelling which contributes to the onset of PE. Several studies show that dNK-cells are associated with PE, as the levels of dNK-cells are higher in PE compared to normal pregnancies. On the other hand, there are also studies that have an opposite conclusion. However, these differences might be due to comparing different parts of the placenta (decidua basalis versus placental bed), but also sample size and analysis methods (Zhang & Wei, 2021).

Not only dNK-cells, but also macrophages turn out to contribute to an impaired spiral artery remodelling. However, it is difficult to study macrophages in PE. Nonetheless, animal studies showed that there is an increased invasion of macrophages in the mesometrial triangle (which correlates to the placental bed in humans) even before the EVT invasion, and thereby impairing spiral artery remodelling. M1 macrophages are mostly observed during PE. M1 macrophages produce pro-inflammatory cytokines, and therefore may play a role in the impaired spiral arteries seen in PE (Faas et al., 2014).

During PE, there is an imbalance between Th-cells, and regulatory T-cells (Tregs). In a healthy pregnancy, Tregs are important for immune tolerance between fetus and mother. What is seen during early onset PE, is an increase in Th1-cells and Th17-cells, and a decrease in Th2-cells and Tregs. Th1-cells and Th17-cells promote inflammation, while Th2-cells and Tregs try to regulate this inflammation. The Th2-cells and Tregs are unable to control the inflammation, thereby contributing to PE (Harmon et al., 2016).

Also DCs are assumed to play a role in the pathogenesis of PE. Women suffering from PE showed increased levels of DC-recruiting chemokines, which indicates that there are more DCs present in the uterus. Overmaturation of DCs caused an upregulated change in the production of pro-inflammatory cytokines, which increased systemic inflammation and maternal injury seen in PE (Wei et al., 2021).

In conclusion, dNK-cells, macrophages, Th-cells and DCs all play a role in early onset PE. This contribution to PE is done via impairing spiral artery remodelling, inflammation and increased levels of chemokines.

Current medications used in PE

There are currently no drugs available to hamper the progression of the disease. However, aspirin, statins, metformin and esomeprazole are being used to prevent the complications seen in PE.

Aspirin belongs to the class of non-steroidal anti-inflammatory drug (NSAID), that inhibits the effects of cyclooxygenases (COX) enzymes (Vane & Botting, 1998). COX enzymes make prostaglandins, which play a role in the generation of inflammatory responses (Ricciotti & FitzGerald, 2011), but this production is inhibited by aspirin. Regarding to PE, aspirin also plays an inhibiting role in the overexpression of hypoxia-induced sFlt-1. Aspirin does this by inhibiting COX-1 (Ma'ayeh & Costantine, 2020). By inhibiting sFlt-1, less symptoms are present. Research showed that low doses of aspirin appeared effective in secondary prevention of PE in high-risk patients, especially in patients with a history of PE. The doses of aspirin should range from 80 to 150 mg, and it should be taken once a day (Atallah et al., 2017). There is evidence that if the dose of aspirin increases, the efficacy also increases. However, the administration of 150 mg aspirin per day needs to be further investigated, as it can inhibit fetal platelet aggregation. Nonetheless, the use of low doses of aspirin appears safe for both mother and fetus (Atallah et al., 2017).

Statins are inhibitors of the 5-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which converts HMG-CoA to mevalonic acid (Ma'ayeh & Costantine, 2020). Recent research showed the importance of statins in PE, as they are believed to increase the production of PlGF, and reduce sFlt-1. The actions of statins include increased EVT invasion, and an improved placental blood flow (Ma'ayeh & Costantine, 2020). The use of statins during pregnancy appears to be safe, as there is no link between statins and stillbirth (Vahedian-Azimi et al., 2021). However, studies show conflicting results as the outcomes of these studies differ. One study included a cohort of women with poor obstetrical history. When these women were given statins, it led to a better pregnancy and even better neonatal outcomes. Another study used a randomized double-blind placebo-controlled trial, and did not show any advantages of using statins. However, this result could be due to a small sample size, so no significant conclusion can be drawn here (Ma'ayeh & Costantine, 2020).

Metformin is a biguanide used mainly as an anti-diabetic agent (Ma'ayeh & Costantine, 2020), and its use during pregnancy is well-established. In pregnancy, metformin showed to inhibit the effects of sFlt-1 and sEng, thereby inhibiting their antiangiogenic effects. Metformin may reverse the placental perfusion and imbalance of (anti)angiogenic factors seen in PE. The clinical data of the effectiveness of metformin for PE varies, as in a double-blinded placebo-controlled trial metformin resulted in a reduction of the incidence of PE, while five randomized trials did not conclude this finding (Ma'ayeh & Costantine, 2020). The difference could be due to physical differences between the mothers. In the double-blinded placebo-controlled trial, pregnant women with a BMI > 35 were taken into account, while this was not the case in the five randomized trials. Due to physical differences, the actions of metformin during PE may differ. However, to conclude this, further research needs to be done.

Esomeprazole is a proton pump inhibitor and showed in preclinical mouse models, that it inhibits the production of sFlt-1 and sEng, thereby reducing endothelial dysfunction. In these mice, esomeprazole appeared safe to use during pregnancy (Ma'ayeh & Costantine, 2020). However, using esomeprazole in human studies was less promising, as there was no significant reduction in sFlt-1 (Cluver et al., 2018).

In conclusion, several medications are being used, but their effectiveness in PE is still debateable. Some studies showed promising results by using aspirin, statins, metformin and esomeprazole as possible drugs for hampering the progression of the disease. However, other studies showed contradictory results. Because of this difference, further research into these medications are necessary.

New possible therapies

Several treatments show promising results for preventing the complications seen in PE. These treatments include esomeprazole, toll-like receptor (TLR) antagonists, placenta-derived mesenchymal stem cells (MSCs), PlGF and relaxin. Their potentials will be discussed below.

The working mechanism of esomeprazole offers opportunities for treatment of PE, because esomeprazole inhibits the production of sFlt-1 and sEng. However, human studies showed that administering 40 mg of esomeprazole, resulted in no reduction in the overexpression of sFlt-1 (Ma'ayeh et al., 2020). Further research can focus on administering higher doses of esomeprazole, or combine it with drugs with the same working mechanism, such as metformin. Importantly, the medication use should maintain safe doses. Adding to this, the study of Ma'ayeh et al (2020) focussed on administering esomeprazole to pregnant women between 26 and 31 week gestation, which resulted in no reduction of sFlt-1. Further research can therefore focus on administering esomeprazole in an earlier stage of pregnancy, instead of a later stage in pregnancy (Ma'ayeh et al., 2020).

Another interesting candidate for treating PE may be toll-like receptor (TLR) antagonists, as these are already used in clinical trials in other diseases, including hypertension. Women suffering from PE also have hypertension. TLR-antagonists can therefore be used to prevent the hypertension seen in PE, and hamper the symptoms of PE. Unfortunately, it is not known if TLR antagonists are safe to use during pregnancy. Therefore, further research is needed in pre-clinical models to investigate if it is a safe treatment to use during pregnancy (Aneman et al., 2020).

Placenta-derived mesenchymal stem cells (MSCs) have potential for further research, as they decrease the ischemic conditions seen in PE. This was done via differentiating human pluripotent stem cells, into multi-nucleated syncytiotrophoblasts. Cytotrophoblasts are stem cells for syncytiotrophoblasts, and they play, as mentioned before, an important role in the invasion of the uterine spiral arteries (Wang, 2010). Due to research in animal models, it is known that MSCs are adult stem cells and have anti-inflammatory and repair potential. Research also showed that MSCs improve the impaired spiral artery remodelling. However, further research into the use of regenerative therapy are needed, and will hopefully lead to successful approaches for the management of PE (Jena et al., 2020).

sFlt-1 plays an important role in early onset PE, and treatments focussing on sFlt-1 might be interesting. A ligand of sFlt-1 is VEGF121, and this has been tested as a possible treatment for PE. Research showed promising results, such as a lower blood pressure and preserved renal function. Besides VEGF121, the role of PlGF has been investigated, as both VEGF121 and PlGF are ligands of

sFlt-1. PlGF treatment is preferred, as PlGF does not bind to VEGF-receptor 2 (VEGFR2), thereby not inducing vascular permeability. Treatment with PlGF in rodent models showed a decreased blood pressure and sFlt-1 expression. However, it is not known yet if PlGF treatment is effective in human placentas (Phipps et al., 2019).

Another possible treatment for PE is relaxin. Relaxin is a hormone secreted by the corpus luteum. In non-pregnant animals, relaxin decreases the blood pressure. Added to this, relaxin also has vasodilatory properties (Pereira et al., 2021). Relaxin has been tested in rodent studies, showing indeed a lower blood pressure in rats with PE, compared to PE controls (Phipps et al., 2019). However, it remains to be investigated whether relaxin has similar effects in humans.

Overall, several possible therapies exist, including esomeprazole, TLR-antagonists, MSCs, VEGF121 and PlGF and relaxin, but further research on these therapies is needed to see whether it can prevent the adverse effects seen in PE.

Discussion

Preeclampsia (PE) is a hypertensive disorder during pregnancy, seen after 20 weeks of gestation. PE can either have an early onset, which is before 34 weeks of gestation, or a late onset, 34 weeks of gestation or later (Marín et al., 2020). Hypertension seen in PE is mostly characterized with proteinuria, but can also occur with maternal organ dysfunction, or uteroplacental dysfunction (Bokslag et al., 2016). The exact ethiology of PE is still unknown, although several hypotheses are given. This thesis focussed on the factors involved in early onset PE, and new possible treatments.

Several soluble factors are associated with PE, such as VEGF or antiangiogenic factors, including sFlt-1, and sEng. Thereby, several processes are also associated with PE, including abnormal placentation and oxidative stress. There are several hypotheses about the ethiology of PE, but the exact pathophysiology is still poorly understood. One hypothesis states that PE happens due to abnormal placentation. Abnormal placentation results from placental hypoxia, which on its turn hampers the production of PlGF. PlGF is needed for development and maturation of the placental vascular system but this is inhibited in PE (Chau et al., 2017). sFlt-1 increases when the levels of PlGF are low. sFlt-1 regulates, in healthy pregnancy, an appropriate depth of the placenta into the uterine wall (Jena et al., 2020). However, during PE, there is an overexpression of sFlt-1, leading to endothelial dysfunction. sEng contributes, together with sFlt-1, to endothelial dysfunction. sEng is also overexpressed in PE, leading to dysfunction. sEng is enhanced by oxidative stress which causes an imbalance between pro-oxidant factors, and anti-oxidant factors, resulting in ROS (Margioula-Siarkou et al., 2022). ROS results in endothelial dysfunction by the release of immune cells (Harmon et al., 2016).

A schematic overview of all these processes can be seen in figure 6.

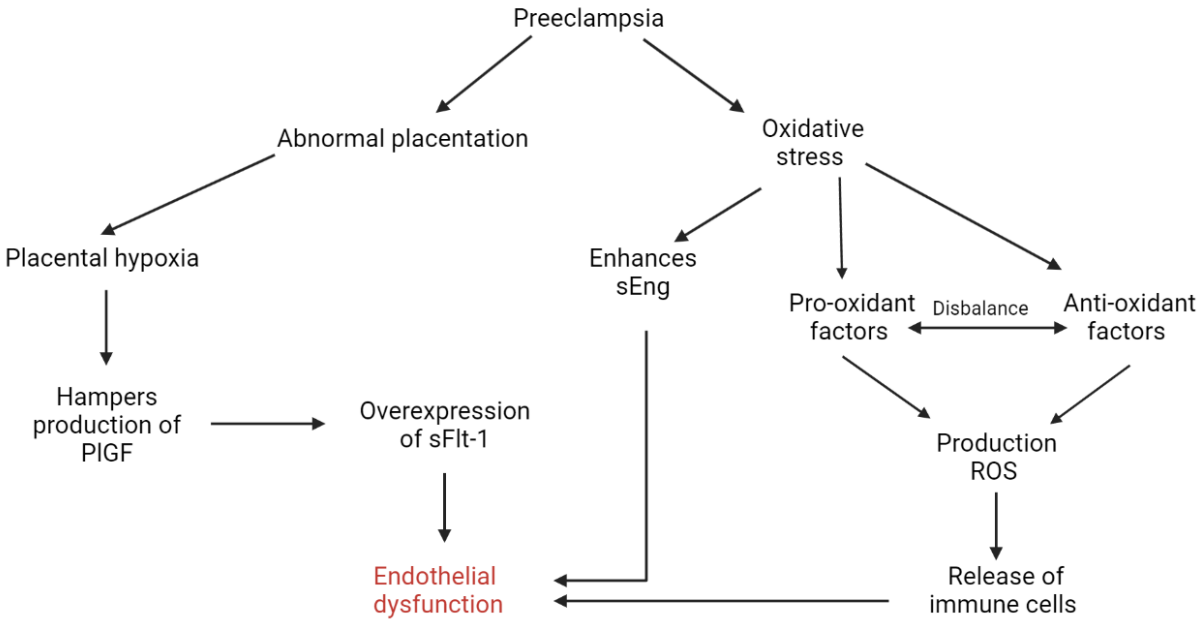

Figure 6. An overview of all processes contributing to the early onset of PE.

Besides the soluble factors, immune cells also play a role in PE. These immune cells include dNK-cells, macrophages, Th-cells and DCs. It is believed that dNK-cells play an important role in spiral artery remodelling , by producing chemokines and cytokines. In PE, there is an impaired dNK-cells function, leading to an impaired spiral artery remodelling (Zhang & Wei, 2021). Other immune cells that contribute to an impaired spiral artery remodelling, are macrophages. Most macrophages in PE produce pro-inflammatory cytokines, contributing to the ethiology of PE (Faas et al., 2014). Th-cells have a different function than dNK-cells and macrophages. In a healthy pregnancy, Th2 and Tregs try

to regulate the inflammation seen in pregnancy. However, in PE, the Th2 and Tregs-cells are unable to control the inflammation, leading to PE (Harmon et al., 2016). The role of DCs in pregnancy is still somewhat unclear, but there is an increase in expression of DCs in PE, indicating that they might play a role (Wei et al., 2021). Further research can focus on DCs more, to investigate the exact role they play, and anticipate on the working mechanism for a possible treatment.
Finding a way to prevent the working mechanisms of the immune cells in PE is key, as this contributes to a healthy pregnancy, and therefore hampers the progression of PE.

For now, early delivery is being used to decrease the complications seen in PE. Aspirin, statins, metformin and esomeprazole are also currently used as medications (Ma'ayeh et al., 2020). However, these drugs do not hamper the progression of the disease. Therefore, further research into new therapies is needed. New therapies can include higher doses of esomeprazole and the combination of esomeprazole and metformin, as both these medicines inhibit the overexpression of sFlt-1 and sEng resulting in a reduction of endothelial dysfunction (Ma'ayeh et al., 2020). However, further research is necessary to investigate if this is safe to use during pregnancy and what doses should be applied. TLR-antagonists can be used to hamper the hypertension seen in PE, thereby contributing to decreasing the development of PE (Aneman et al., 2020). Another interesting therapy includes MSCs, as these are believed to have repair potential, and anti-inflammatory properties. Next to this, MSCs are also believed to improve the impaired spiral artery remodelling seen in PE (Jena et al., 2020). PlGF and relaxin are also possible treatments, as PlGF decreases the overexpression of sFlt-1, and relaxin lowers blood pressure, thereby reducing the risk for hypertension (Phipps et al., 2019). All of the above mentioned treatments need to be investigated more in animal models, before we test it in humans. Further research should investigate whether the treatments hamper the progression of the disease, and if it is safe to use during pregnancy. Furthermore, the most important factors in PE are sFlt-1 and sEng. Therefore, further research should aim to inhibit their overexpression, by, for example, inhibit the receptor they bind on. This will hopefully reverse the adverse effects seen in PE.

In conclusion, new treatments are necessary to hamper the progression of PE. This is still difficult to accomplish, as the ethiology of PE is not fully understood yet. However, PlGF, sFlt-1, sEng and immune cells are all believed to play an important role in the ethiology of PE. Focus of further research should be on TLR-antagonists, MSCs, PlGF and relaxin to hamper the progression of PE. Also, preventing the overexpression of sFlt-1 and sEng, as these are important factors in PE, can hopefully adverse the effects seen in mother and child suffering from PE.

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