CHAPTER I

GENERAL INTRODUCTION

Introduction

PREECLAMPSIA – A HETEROGENEOUS DISEASE WITH SERIOUS CONSEQUENCES Preeclampsia is a pregnancy-specific syndrome that is characterized by endothelial dysfunction, presenting clinically as hypertension and proteinuria after 20 weeks of gestation. The syndrome can be accompanied by a variety of complications including headache, visual disturbances, epigastric pain, generalized seizures (eclampsia) and HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome.[1;2] Because the etiology of preeclampsia is largely unknown and probably as heterogeneous as its presentation, accurate prediction, prevention, and rational therapy of pre-eclampsia are still not possible. The only cure for preeclampsia is delivery—often preterm—which rescues the mother but frequently compromises fetal outcome. Preeclampsia is not uncommon, complicating 2-8% of pregnancies, and therefore contributes significantly to maternal and fetal morbidity and mortality worldwide.[3] In addition, the burden of preeclampsia is not limited to the period around pregnancy; the condition is associated with an increased prevalence of cardiovascular risk factors later in life, in both mothers and children.[4-6]

PREECLAMPSIA AND THE PLACENTA Although the exact causes of preeclampsia are still unidentified, the condition is believed to originate in the placenta, as evidenced by the fact that the placenta alone, not the fetus, is required for the development of preeclampsia.[7-9] The placenta is a newly formed organ, composed of both fetal and maternal tissues. The placenta supports life throughout the gestational period of a developing fetus by supplying oxygen, protective antibodies, and nutrients while eliminating waste products. Preeclampsia is frequently characterized by disturbed placental development, which is already observable during the early stages of pregnancy. In a subset of preeclamptic women, however, aberrant placental development is a less prominent feature. Strikingly, the extent of placental dysfunction is related to

an earlier onset of preeclampsia and therefore, preeclampsia is often subdivided into early-onset and late-onset preeclampsia.[10] Early-onset preeclampsia is considered a disorder in which abnormal placental morphology, reduced placental volume, intra-uterine growth restriction, and adverse maternal and neonatal outcomes are prominent features.[10-13] Late-onset preeclampsia, on the contrary, is considered a maternal disorder. It is often associated with a normal placental morphology, larger placental volume, normal fetal growth, and more favorable maternal and neonatal outcomes.[10;11;13]

IMMUNOLOGIC, GENETIC AND ANGIOGENIC ASPECTS OF PREECLAMPSIA A variety of risk factors have been identified that predispose women to preeclampsia. The most significant risk factors for developing preeclampsia are a history of preeclampsia and the presence of antiphospholipid antibodies.[14] Other important risk factors for preeclampsia include nulliparity, a family history of preeclampsia, maternal age ≥ 40, obesity and several underlying medical conditions, including pre-existent hypertension, pre-existent diabetes and autoimmune disease.[1;14] In these epidemiological data lays the clue why this thesis is focused towards the immunologic and genetic aspects of preeclampsia; the strong relation between preeclampsia and autoimmune disorders supports a role for the immune system in preeclampsia. Likewise, the clear familial component that characterizes preeclampsia suggests that genetic factors contribute to the development of preeclampsia.

In the first part of this introductory chapter (*Part 1 - Placental development)*, the development and function of the placenta during normal pregnancy will be explained. The second part (*Part 2 - Preeclampsia)* focuses on the pathophysiology of preeclampsia. This section will go into depth by focusing on two putative causes of preeclampsia: the presence of genetic variants and dysregulation of the complement system. In addition, the consequences of placental dysfunction in the setting of preeclampsia will be described,

including angiogenic dysregulation and the subsequent generalized endothelial dysfunction.

The third part of this chapter will focus on the research questions that form the basis for the experiments and the studies described in this thesis (*Part 3 - This thesis*). Finally, an outline of the chapters will be given.

Part 1 - Placental development

IMPLANTATION Shortly after conception, the placenta starts to develop in the uterus. However, before the fertilized ovum reaches the uterus, the lining of the uterus has already undergone a variety of important changes. The thickness and the vascularity of the uterine lining have increased, and the uterine glands have become elongated. Furthermore, the tissue between the uterine glands now contains a great number of large round, oval or polygonal cells, termed decidual cells. At the time all these changes have taken place, the lining of the uterus is termed the decidua.

On reaching the cavity of the uterus, the fertilized ovum—now termed the blastocyst—adheres to the decidua. This is the site where the placenta will subsequently develop. The attached blastocyst excavates a cavity in the decidua, in which it becomes embedded. The structure of the blastocyst that is responsible for this process of excavation is termed the trophoblast. The trophoblast proliferates rapidly and forms a network of branching projections —the chorionic villi—which infiltrate the uterine decidua and absorb from it the nutrients for the growth of the embryo (Figure 1).[15]

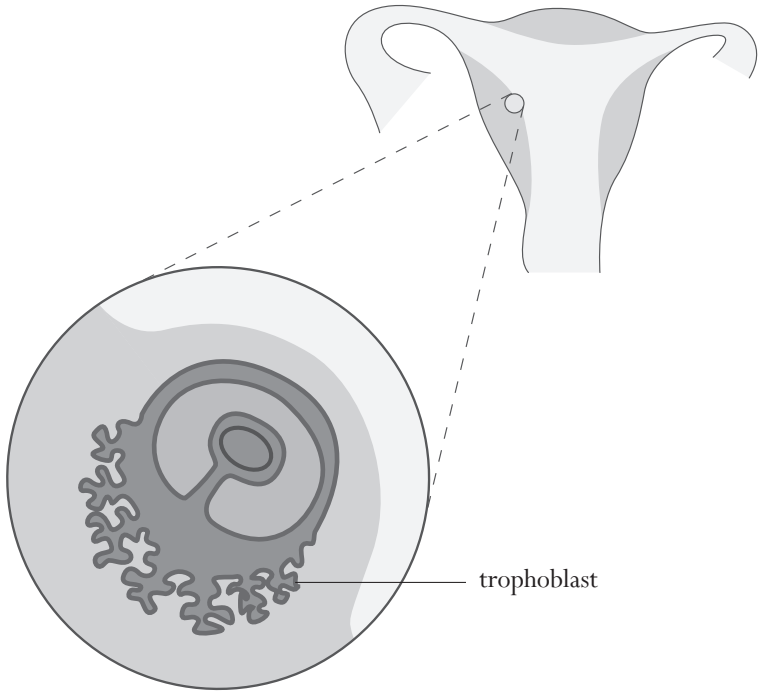

Figure 1: Implantation and trophoblast development

THE PLACENTA FORMS THE FETAL-MATERNAL INTERFACE

While infiltrating the maternal tissues, the trophoblast grows into the maternal blood vessels that are present within the uterine wall. The invasive phenotype of trophoblast cells enables them not only to invade but also to remodel the uterine spiral arteries. As a result, these uterine vessels change from narrow, muscular arterioles into flaccid, high-capacity vessels of low resistance, which fill the spaces in the trophoblastic network with maternal blood.[16;17] All the spaces in the trophoblastic network—which communicate freely with one another—together form the intervillous space. The decidua and the intervillous space together form the maternal portion of the placenta (Figure 2).

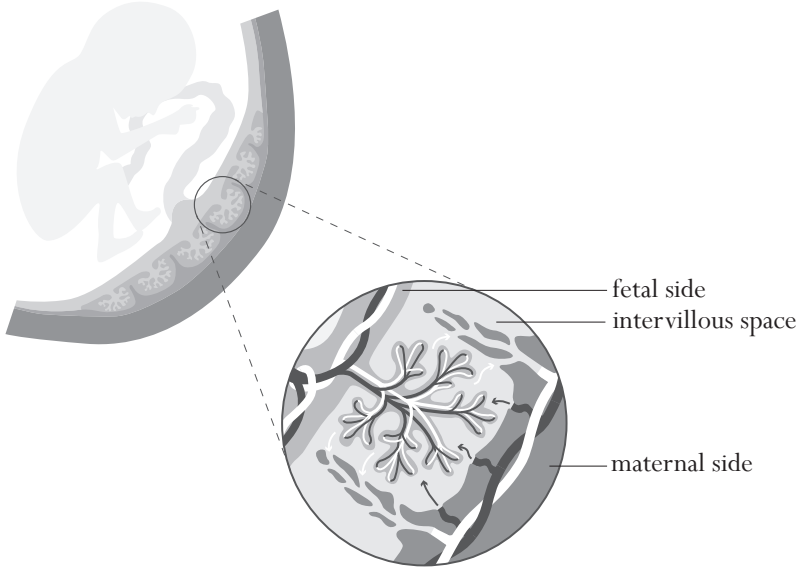

Figure 2: The fetal and maternal portions of the placenta. The fetus and the mother are in direct contact with each other but their blood currents are separated from one another by the delicate walls of the villi.

The chorionic villi are of fetal origin and therefore they make up the fetal portion of the placenta. These villi branch repeatedly and as a result, the fetal part of the placenta has an enormous surface area. During the course of pregnancy, the chorionic villi increase in size and become vascularized. The great majority of the villi float freely in the intervillous space, being surrounded by maternal blood. All villi are entirely covered by syncytiotrophoblast, which therefore forms the continuous physical barrier between mother and fetus, also called the fetal-maternal interface. So throughout pregnancy, the placenta is composed of a fetal portion and a maternal portion that are in direct contact with each other (Figure 2).

EXTENSIVE CONTACT FACILITATES THE MAIN FUNCTIONS OF THE PLACENTA Although both the fetal and the maternal blood currents traverse the placenta, their blood does not intermingle. However, oxygen, nutrients and protective antibodies from the maternal blood can be transported to the fetal circulation. At the same time, the placenta receives the waste products from the fetus and discharges them into the maternal circulation for disposal. The large surface area of all villi together facilitates this exchange of nutrients, waste products and protective antibodies between mother and fetus.

EXTENSIVE CONTACT ALSO IMPOSES AN IMMUNOLOGICAL CHALLENGE Although the large surface area of the placenta facilitates the main functions of the placenta, the extensive contact between mother and fetus also imposes an immunological challenge. As early as 1953, Peter Medawar put forward the idea of the fetus as a semi-allograft; the trophoblast is derived from the fetus and so it expresses paternal antigens which the mother's immune system may recognize as foreign.[18] These paternal antigens can consequently elicit an immune response, possibly leading to fetal rejection if the maternal immune response is not adequately regulated. Therefore, in order to prevent maternal anti-fetal rejection, an immune privileged site must be created at the fetal-maternal interface. Trophoblasts have several characteristics that contribute to the prevention of both cellular and humoral maternal immune responses, together creating this immune privileged site.

INHIBITING IMMUNE MECHANISMS WITHIN THE PLACENTA Cellular immune mechanisms are prevented in several ways. Importantly, trophoblast cells do not express the classical HLA-A, HLA-B, HLA-DR, HLA-DQ, and HLA-DP molecules that are the main targets for alloreactive T-cells in the setting of organ transplantation. However, they do express HLA-C, HLA-E, and HLA-G molecules by which they can avoid cell-mediated cytotoxicity.[19;20] This is illustrated by the observation that HLA-

G-expressing cells induce regulatory T-cell activity and avoid NK-mediated cytotoxicity.[21;22] T cell activity is furthermore suppressed by the expression of indoleamine-2,3-dioxygenase (IDO) on the trophoblast membrane.[23]

Although cellular immune mechanisms have been subject of extensive investigation, less is known about the role of humoral immunity and the interplay with the complement system during pregnancy. Trophoblast expresses fetal antigens to which maternal lymphocytes are exposed and to which maternal B-cells can form targeted antibodies. The presence of anti-HLA or anti-paternal antibodies is indeed observed in approximately 30% of pregnant women, but it is normally not associated with pregnancy complications.[24] A possible explanation for this phenomenon is that trophoblast is well prepared for maternal anti-fetal antibody-mediated attack. A mechanism by which trophoblasts can repress humoral immune mechanisms is the expression of complement regulatory proteins on their cell membrane. Indeed, all three membrane-bound complement regulatory proteins (DAF, MCP and CD59) are highly expressed on trophoblast cells, strategically positioned at the fetal-maternal interface to protect the fetus from maternal immune responses.[25;26] Because a large part of this thesis deals with complement activation at the fetal-maternal interface, an introduction of the complement system will be given below.

THE COMPLEMENT SYSTEM: BALANCE BETWEEN ACTIVATION AND REGULATION The complement system is an essential component of the human immune system.[27;28] It is composed of over 30 soluble- and membrane-bound proteins that are constantly in a 'controlled active state', which means that the system is ready for action at all times. Specific triggers, such as invading pathogens, can activate the complement system by setting off a cascade of enzymatic reactions and positive feedback loops. The complement cascade is capable of achieving enormous amplification, as an initially small number of activated complement molecules produced early in the cascade

eventually leads to the generation of a large number of effector molecules. The result is destruction of potentially dangerous targets within seconds. The complement system also provides a link between innate and adaptive immune systems, thereby further facilitating the removal of potentially dangerous pathogens. Although the main role of the complement system is to protect the host against invading pathogens, the system is also involved in tissue regeneration and clearance of necrotic and apoptotic cells.[29]

WHAT CAUSES ACTIVATION OF THE COMPLEMENT SYSTEM? The complement system (Figure 3) can become activated by three distinct pathways: the classical, the lectin and the alternative pathway.[27] Each pathway has specific triggers and recognition molecules by which it can become activated. Although the three pathways differ in how they are activated, they do share a final common pathway. All pathways converge at the level of C3. From this point, a final common pathway leads to the effector functions of complement, namely chemotaxis, opsonization and cell lysis. The classical pathway of complement is activated when its recognition molecule C1q binds to immune complex deposits, antibody-antigen binding, charged molecules, and apoptotic and necrotic cell debris. This pathway links innate immunity to adaptive B-cell mediated immunity after an antibody response has developed.[28] However, this pathway also forms an important threat in the setting of autoimmune diseases and transplantation, as deposition of auto- or allo-antibodies can activate the classical pathway leading to complement-mediated organ damage or graft rejection. The lectin pathway is activated when mannose-binding lectin (MBL) or ficolins recognize carbohydrates that are present on the surface of a wide range of pathogens. The recognition molecule of the lectin pathway is MBL. However, further downstream the pathway follows the same steps as classical complement activation, via C2, C4 and the final common pathway. Consequently, the lectin pathway is functionally similar to the classical pathway and it can be challenging to distinguish between

classical and lectin pathway activation. Finally, the alternative pathway is initiated spontaneously. The pathway is characterized by continuous hydrolysis of C3, and, at the same time, tight regulation to prevent uncontrolled activation. Activation of the alternative pathway can be considered as a positive feedback loop, as it amplifies complement activation initiated by the classical- or lectin pathway. Whenever C3 is formed by the classical- or lectin pathway activation, factor B and D immediately bind to it. This newly formed complex is subsequently stabilized by properdin, forming an enzyme that leads to more C3 activation. Thereby, a positive feedback loop is created, leading to instant amplification of the inflammatory reaction initiated by classical or lectin pathway activation.[30]

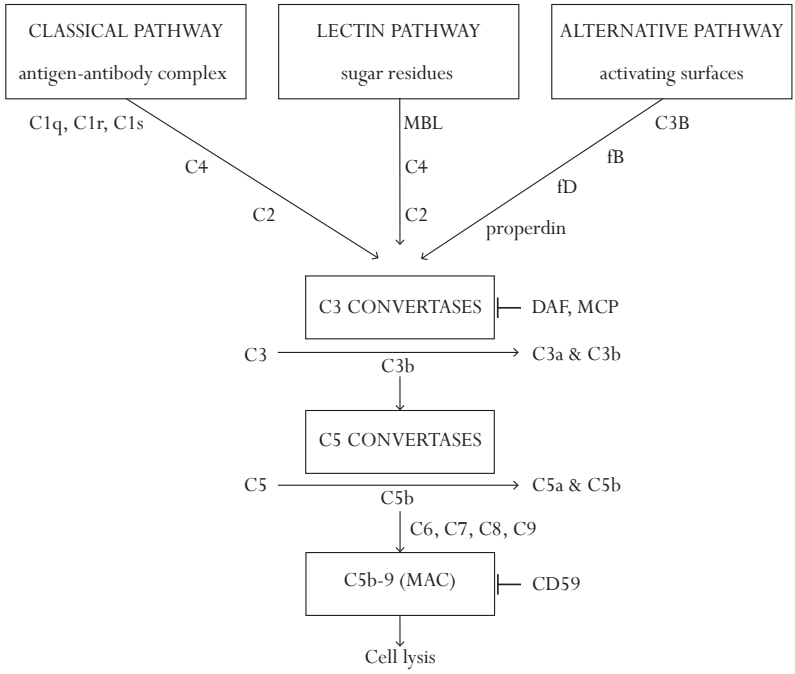

Figure 3: A schematic overview of the complement system

WHAT ARE THE EFFECTS OF COMPLEMENT ACTIVATION?

Activation of the complement cascade has various effects, which all have the purpose to start eliminating pathogens in the time before the adaptive immune system is ready for action. Firstly, complement activation results in the generation of the potent anaphylatoxins C3a and C5a, which attract neutrophils and monocytes to the site of inflammation. Aside from chemotaxis, these anaphylatoxins also cause vasodilatation, smooth muscle contraction, histamine release from mast cells, and oxidative burst from neutrophils.[31;32] Secondly, the cleavage of certain complement proteins results in the generation of split products that coat the surface of target cells and pathogens, thereby facilitating the process of phagocytosis. Finally, complement activation can lead to the formation of the membrane attack complex (MAC). The MAC is a complex of proteins that work together to create a pore-like structure on cell membranes. Insertion of this MAC into cell membranes leads to cell lysis and, as a result, cell death. Importantly, even sublytic quantities or partial membrane attack complexes—which do not lead to cell death—are important for signaling effects.[33;34]

HOW IS THE COMPLEMENT SYSTEM REGULATED?

Complement components are not capable of distinguishing foreign agents from self-tissues, and therefore the system operates rather non-specifically. This characteristic makes complement a potentially dangerous system for the host, as complement components have the capacity to bind and damage the host's own tissues. To prevent complement-mediated damage to host cells, tight regulation is necessary. Indeed, a complex array of protective mechanisms exists, illustrated by the fact that nearly half of all complement components have a regulatory function. Soluble and membrane-bound complement regulators act in concert to protect cells and tissues from unintended complement-mediated injury.[35] Their goal is to prevent damage to host tissues by inhibiting the cascade on three vital points: activation, amplification and the formation of

the membrane attack complex. The membrane bound regulatory proteins include CD59, Decay Accelerating Factor (DAF) and Membrane Cofactor Protein (MCP).[36] These factors are widely expressed by host tissues to prevent complement from inducing auto-injury. CD59 binds to components of the MAC, preventing its assembly and, therefore, its insertion into the cell membrane. DAF prevents the formation and accelerates the destruction of C3-convertases, which are enzymes derived from C4 that normally lead to C3 activation and the formation of the anaphylatoxins C5a and C3a. MCP binds the complement components C3b and C4b and facilitates their degradation by the soluble complement regulator factor I, thereby preventing further activation and amplification of the complement cascade. Tissues that are in direct contact with blood—such as endothelium and placental tissues—are constantly exposed to complement components. As a consequence, these tissues need to express many different complement regulatory proteins to prevent the potentially deleterious effects of complement activation. Soluble regulatory proteins are factor I, factor H, and the C4-binding protein. These soluble regulators mainly affect enzymes involved in the alternative pathway, thereby preventing amplification of the cascade and blocking positive feedback loops.

When the complement system manages to damage host tissues, the extent of activation has either exceeded the host's regulatory capacity, or the host's regulatory mechanisms worked inadequately. Excessive activation could result from an overload of immune complexes, for instance in the setting of autoimmune diseases, in which auto-antibodies result in the generation of immune complexes by binding antigens on host tissues. Constant exposure of host tissues to auto-antibodies may overwhelm complement regulatory mechanisms, thereby allowing direct complement-mediated injury. The consequences of inadequate regulation can be observed in patients with mutations in their genes coding for complement regulatory proteins, for instance patients with hemolytic uremic syndrome[37] but also in women with the HELLP syndrome as well as preeclampsia.[38;39]

WHAT IS THE ROLE OF COMPLEMENT IN PHYSIOLOGICAL PREGNANCY? Although the presence of complement deposits is generally a pathologic finding, complement split products are frequently observed in placentas from healthy women with uncomplicated pregnancies. Limited amounts of both early (C1q and C4) and late (C5, C6, C9, MAC) complement components have been demonstrated in normal placentas.[40-44] In addition, serum levels of C3, C4 and total hemolytic complement (CH50) gradually rise with 10-50% during physiological pregnancy, likely due to increased synthesis.[45] Furthermore, plasma concentrations of anaphylatoxins and other complement activation products are significantly higher in normal pregnant women as compared to non-pregnant women.[46;47] In conclusion, low grade complement activation is compatible with successful pregnancy.

Importantly, placental complement deposits are not accompanied by widespread tissue damage. This absence of tissue injury can be explained by the extensive expression of complement regulatory proteins at the fetal-maternal interface.[25;26] Murine studies have underscored the importance of complement regulation during pregnancy. Embryos deficient in Crry—a murine complement regulator—are characterized by compromised survival, and their placentas show marked C3 depositions and inflammation. Mice deficient in both Crry (Crry-/-) and C3 (C3-/-) are rescued from lethality[48], which elegantly demonstrates that complement activation at the fetal-maternal interface causes fetal loss. These observations also underline the critical role of complement regulation in successful pregnancy.

Importantly, it has been suggested that the classical pathway component C1q contributes to trophoblast invasion of the decidua. Invading trophoblasts actively synthesize C1q, which promotes trophoblast adhesion and migration.[49;50] Defective local production of C1q may be involved in pregnancy disorders that are characterized by impaired trophoblast invasion, such as preeclampsia.[51]

Part 2 - Preeclampsia

WHAT IS THE ROLE OF THE PLACENTA IN PREECLAMPSIA?

The pathophysiology of preeclampsia is generally subdivided into two stages.[52] The early, preclinical stage is associated with abnormal placental development, whereas the late, clinical stage is characterized by widespread endothelial dysfunction and dysregulation of angiogenic factors. During the early stage, the mother has not yet developed the typical manifestations of preeclampsia. However, profound changes can already be observed in the placenta. During normal pregnancy, the trophoblast acquires an invasive phenotype and remodels the uterine spiral arteries to assure sufficient placental perfusion.[16;17] In preeclampsia, on the contrary, remodeling of the uterine spiral arteries into flaccid, high capacity vessels fails (Figure 4).[53-55] The result is increased uterine vascular resistance and diminished placental perfusion. In a subset of women destined to develop preeclampsia, these changes can be detected long before the onset of the clinical syndrome.[56-58] However, poor placental development should not be regarded as the cause of preeclampsia but rather as a powerful predisposing factor. Importantly, not all pregnancies characterized by poor placentation have adverse outcomes.[59] Moreover, not all women with preeclampsia have profound placental dysfunction.

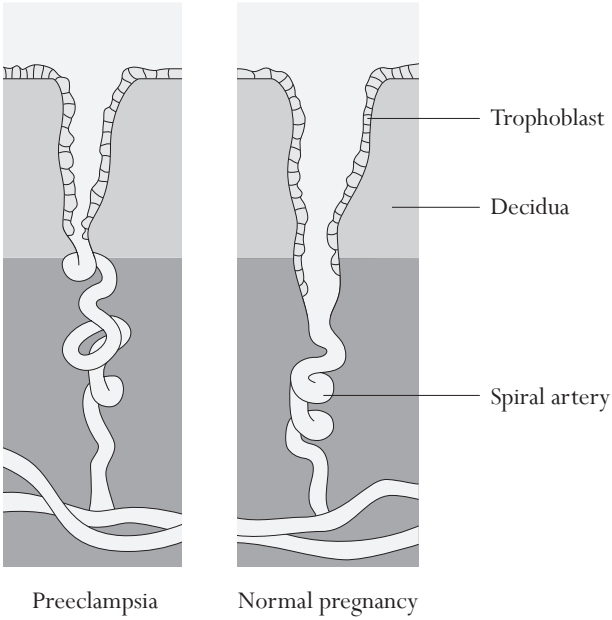

Figure 4: During normal pregnancy, the trophoblast acquires an invasive phenotype and remodels the uterine spiral arteries to assure sufficient placental perfusion. In preeclampsia, on the contrary, remodeling of the uterine spiral arteries into flaccid, high capacity vessels fails.

LATE-ONSET PREECLAMPSIA; A RESULT OF MATERNAL SUSCEPTIBILITY? In case of late-onset of preeclampsia—in which placental dysfunction is less prominent—maternal constitutive factors likely contribute to the pathogenesis of preeclampsia. Important risk factors for developing preeclampsia include a patient history of preeclampsia as well as a family history of preeclampsia, suggesting that preeclampsia may have a genetic background. Similarly, conditions characterized by endothelial dysfunction increase the risk for developing preeclampsia, suggesting that maternal cardiovascular and metabolic syndrome-like disorders contribute to the pathogenesis of preeclampsia.[1]

Evidence for a genetic component of preeclampsia comes from family studies, which have shown that primigravid women with a family history of preeclampsia have a two- to five-fold increased risk of developing preeclampsia as compared to primigravid women with no such history.[60-64] Additionally, the risk of preeclampsia is increased more than seven-fold in women who have suffered from preeclampsia in any previous pregnancy.[14] Interestingly, paternal genetic components also predispose to preeclampsia, which is illustrated by the fact that fathers who were born from a preeclamptic pregnancy, have a greater chance of fathering a child who is also born from a preeclamptic pregnancy.[65;66] Because the paternal genes are passed on to the fetus, this finding indicates that the genotype of the fetus contributes to the overall risk of preeclampsia. Additionally, there are marked differences in the incidence of preeclampsia among various ethnic groups, which further supports a role for a genetic component in the pathogenesis of preeclampsia.[1] In conclusion, epidemiologic data suggest that both maternal and paternal genes contribute to the development of preeclampsia. However, the underlying genetics are complex, and it is currently unclear which genes are involved and how individual genetic variants contribute to preeclampsia. Therefore, numerous genetic association studies have been performed to elucidate the genetic background of preeclampsia. These genetic association studies have mainly focused on so-called "candidate genes" which are genes that are likely to be involved in the pathogenesis of preeclampsia. Despite a sharp increase in the number of published studies regarding genetic associations in preeclampsia, attempts to replicate findings from these studies have frequently yielded inconsistent results. This lack of reproducibility can be due to population diversity, but it is often the result of small sample sizes or false-positive results.[67] Because the prior probabilities of genetic associations are low, the number of false-positive associations that are generated by chance alone is high. Therefore, replicating an association in independent studies is essential for identifying true genetic associations among the large number of false-positives.

As previously mentioned, women suffering from pre-existent hypertension, diabetes, renal disease and obesity have a significantly higher risk for developing preeclampsia. Importantly, the genes that predispose to the aforementioned conditions could indirectly also predispose to preeclampsia. For instance, certain genetic variants may increase the risk for developing hypertension, which in turn causes an increased susceptibility for developing preeclampsia. Such predisposing disorders could set off a cascade of placental and systemic inflammation as well as oxidative stress, resulting in late onset pre-eclampsia.[1;52]

PLACENTAL DYSFUNCTION IN PREECLAMPSIA: AN IMMUNOLOGICAL PROBLEM? As opposed to late-onset preeclampsia, early-onset preeclampsia is frequently characterized by reduced placental perfusion and marked histopathological changes in the placenta, including compromised villous volume and surface area.[11;13;58] It has been proposed that an excessive or atypical maternal immune response to trophoblast underlies this aberrant placental development in the setting of preeclampsia.[59;68] Evidence for immune dysregulation in the pathophysiology of preeclampsia comes from epidemiological data, which have pointed out that preeclampsia is relatively common among women with the autoimmune diseases antiphospholipid syndrome (APS) and systemic lupus erythematodes (SLE). The presence of anti-phospholipid antibodies increases the risk for developing preeclampsia nine-fold.[14] Conversely, among women who have had preeclampsia, anti-phospholipid antibodies are more frequently found.[69] In women with APS/SLE, pregnancies are frequently complicated by recurrent miscarriage, intra-uterine fetal loss and preeclampsia, and the complement system often plays an essential role in mediating these pregnancy complications.[70] The relation between preeclampsia and the aforementioned autoimmune diseases, combined with the important role of the complement system in mediating pregnancy complications in women with APS/SLE, suggests that the complement system may also be involved

in the pathogenesis of preeclampsia in women without underlying autoimmune disease.

WHAT IS THE EVIDENCE FOR COMPLEMENT DYSREGULATION IN HUMAN PREECLAMPSIA? Indeed, several lines of evidence support the idea that complement is involved in the pathogenesis of preeclampsia. In preeclampsia, serum levels of C3a, C4d, and soluble MAC are markedly increased as compared to healthy pregnant women.[47] In addition, women with increased serum levels of the alternative pathway fragment Bb during early pregnancy are more likely to develop preeclampsia later in pregnancy[71], suggesting that early activation of the alternative pathway may play a role in the pathogenesis of preeclampsia. Although the distribution of placental complement components is similar to physiological pregnancy, the quantity of these depositions is generally increased in cases of preeclampsia.[40;44]

A putative mechanism by which the complement system can be activated in the setting of preeclampsia is the presence of circulating (auto)-antibodies. A subgroup of women with preeclampsia have circulating auto-antibodies directed against the angiotensin-I type II receptor (AT1-AA).[72] These antibodies could result in hypertension through stimulating the angiotensin-I receptor. However, these antibodies may also cause complement activation, as demonstrated in a murine model. Importantly, injecting mice with these auto-antibodies results in complement activation as well as angiogenic dysregulation, an important hallmark in the pathophysiology of preeclampsia.[73] It is important to note that not only excessive activation but also inadequate regulation of the complement system could be responsible for complement deposits in women with preeclampsia. Importantly, mutations in complement regulatory proteins predispose to preeclampsia.[39] These mutations have also been found in women with the HELLP syndrome, which is closely linked to preeclampsia.[38]

In summary, increasing evidence suggests that the complement

system is involved in the pathogenesis of preeclampsia. However, relatively little is known about which pathway is activated and how (changes in) complement regulation contribute to the pathogenesis of preeclampsia.

LATE MANIFESTATIONS OF PLACENTAL DYSFUNCTION: ANGIOGENIC DYSREGULATION During the second stage of preeclampsia, the clinical manifestations of the syndrome develop. During this stage, reduced uteroplacental flow and episodes of irregular placental perfusion likely lead to the generation of reactive oxygen species[74], resulting in placental oxidative stress and placental dysfunction.[75] Subsequently, the preeclamptic placenta releases excessive amounts of the anti-angiogenic factors soluble fms-like tyrosine kinase (sFlt-1) and soluble endoglin (sEng).[76-78] The anti-angiogenic factor sFlt-1 is a splice variant of the vascular endothelial growth factor (VEGF) receptor. By sequestering VEGF and placental growth factor (PlGF), sFlt-1 prevents their interaction with their membrane-bound receptors. Endoglin is a co-receptor for transforming growth factor B (TGF-B). Its soluble form, sEng, binds and thereby neutralizes TGF-B. As VEGF, PlGF and TGF-B are all growth factors essential for endothelial integrity, relative shortage of these angiogenic factors results in endothelial dysfunction. Therefore, these anti-angiogenic factors likely cause the generalized endothelial dysfunction that characterizes preeclampsia (Figure 5).[79;80] Indeed, animal models have demonstrated that administration of sFlt-1 and sEng recapitulates the features of preeclampsia.[77;78] Importantly, it is the syncytiotrophoblast—the outer layer of the placental villi—that produces these anti-angiogenic factors. [81]

TROPHOBLAST SHEDDING LIKELY CONTRIBUTES TO THIS ANGIOGENIC IMBALANCE In addition to anti-angiogenic factors, the preeclamptic placenta also releases excessive amounts of syncytiotrophoblast fragments. During normal pregnancy, syncytiotrophoblast fragments are constantly shed into the maternal

circulation but this shedding is markedly increased in preeclampsia.[82] This increase in shedding may be due to oxidative stress and subsequent apoptosis and necrosis of the syncytiotrophoblast.[83] These syncytiotrophoblast fragments release sFlt-1 and remain transcriptionally active up to 48 hours after their release from the placenta.[84] This implies that syncytial fragments may form an important source of sFlt-1 in the maternal circulation and that they could play an important role in causing systemic endothelial dysfunction (Figure 5). The fragments may contribute to endothelial dysfunction, as they inhibit endothelial proliferation *in vitro*.[85]

THE END-RESULT: GENERALIZED ENDOTHELIAL DYSFUNCTION Regardless whether placental dysfunction or maternal susceptibility underlies the development of preeclampsia, the end-result is endothelial dysfunction. Various studies have revealed that preeclampsia is associated with generalized endothelial dysfunction. Firstly, women with preeclampsia are characterized by impaired flow-mediated vasodilation[86;87], as well as by impaired acetylcholine-mediated vasorelaxation.[88] In addition, preeclampsia is associated with an enhanced vascular reactivity to angiotensin II[89] and serum from preeclamptic women induces endothelial activation *in vitro*.[90] Importantly, endothelial dysfunction can explain the clinical manifestations of preeclampsia.[91] Hypertension in the setting of preeclampsia may result from impaired endothelial control of vascular tone, whereas proteinuria could be a manifestation of increased vascular permeability. Headache, seizures, visual disturbances, epigastric pain and fetal growth restriction could result from endothelial dysfunction in the vasculature of target organs, including the brain, liver, and placenta. When expectant management of preeclampsia is no longer possible due to the severity of the aforementioned symptoms, delivery is the only option left. As a result, preeclampsia continues to contribute to maternal and fetal morbidity and mortality worldwide.

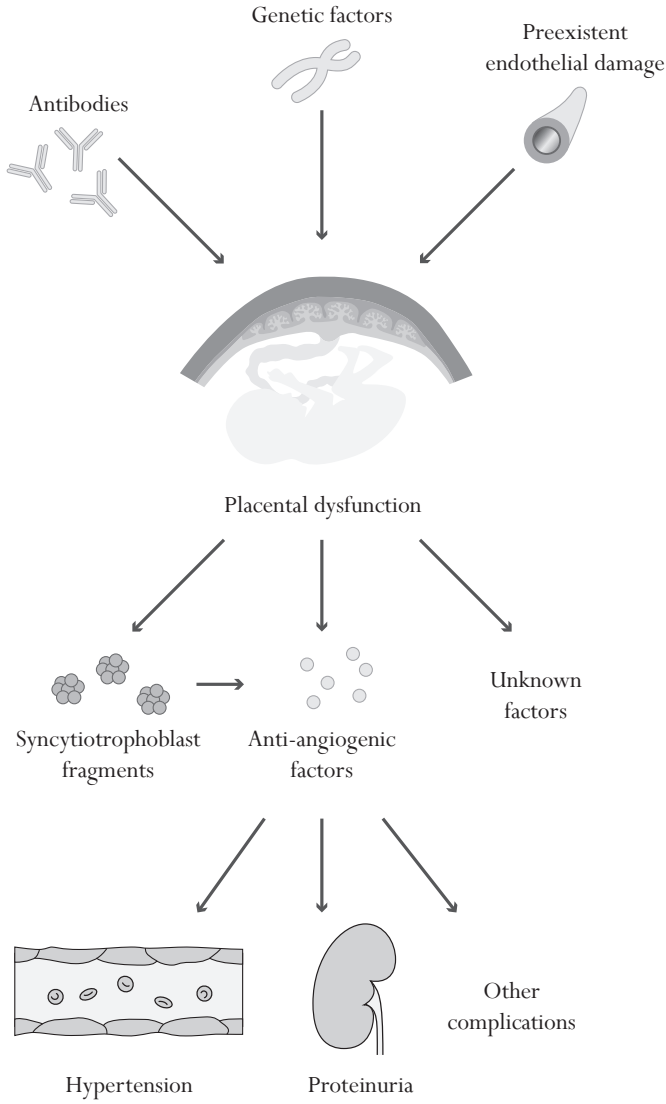

Figure 5: The pathophysiology of preeclampsia. The dysfunctional placenta releases excessive amounts of anti-angiogenic factors and syncytiotrophoblast fragments. These factors lead to endothelial dysfunction, which, in turn, can explain the clinical manifestations of preeclampsia.

Part 3 - This thesis

Despite decades of research, it remains largely unknown what drives placental dysfunction in preeclamptic women. Likewise, it is uncertain what exactly underlies maternal susceptibility in the setting of preeclampsia. Further research into the pathophysiological mechanisms underlying preeclampsia may provide targets for prevention, diagnosis and therapy. This thesis touches upon genetic, immunologic and angiogenic aspects of preeclampsia, and particularly on:

- The contribution of genetic variants in the development of preeclampsia
- Placental complement dysregulation in the setting of preeclampsia and the autoimmune disorders APS and SLE
- The source of sFlt-1 and the spread of transcriptionally active syncytiotrophoblast fragments throughout the maternal body

AIMS OF THIS THESIS

- To determine which genetic variants are reproducibly and significantly associated with preeclampsia
- To determine the role of placental C4d in SLE and antiphospholipid syndrome related adverse pregnancy outcome
- To investigate the relation between placental complement dysregulation and preeclampsia
- To explore the relationship between placental C4d and the extent of placental dysfunction in the setting preeclampsia
- To investigate whether placenta-derived fragments are retained in the maternal lung, and whether they remain transcriptionally active *in situ*

THESIS OUTLINE The role of genetic variants in preeclampsia is explored in **Chapter 2**, in which the results are described of a meta-analysis of genetic association studies that have been performed in the setting of preeclampsia.

Complement activation plays an important role in the pathogenesis of pregnancy complications in the setting of autoimmune disease. In **Chapter 3** the potential of the classical pathway component C4d as a biomarker for pregnancy complications in women with the autoimmune disorders antiphospholipid syndrome and systemic lupus erythematosus is explored.

Preeclampsia is strongly related to the autoimmune disorders APS and SLE. As complement activation plays an important role in mediating pregnancy complications in women with APS/SLE, this strong correlation suggests that the complement system may also be involved in the pathogenesis of preeclampsia. Therefore, **Chapters 4 and 5** focus on the role of placental complement activation in women with preeclampsia. **Chapter 4** describes the study in which we have investigated which complement pathway is activated within the placenta during preeclampsia. **Chapter 5** focuses on the relation between placental complement activation and the extent of placental dysfunction in the setting of preeclampsia.

Chapter 6 is devoted to angiogenic dysbalance in women with preeclampsia. In this chapter, the placental expression of sFlt-1 and the systemic spread of syncytiotrophoblast fragments is evaluated.

Overall, the work described in this thesis focuses on pathogenic mechanisms in the setting of preeclampsia. How do the genetic, immunologic and angiogenic aspects of preeclampsia together contribute to the endothelial dysfunction that is characteristic of preeclampsia? What is cause and what is consequence? These questions are addressed in **Chapter 7**, the general discussion, where the findings of this thesis will be summarized and placed in a more general perspective. The general discussion will be followed by a summary in Dutch.

REFERENCES

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet* 2010; 376:631-644.
2. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol* 2002; 99:159-167.
3. Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. *Lancet* 2006; 367:1066-1074.
4. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ* 2007; 335:974.
5. Davis EF, Lazdam M, Lewandowski AJ, Worton SA, Kelly B, Kenworthy Y *et al.* Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review. *Pediatrics* 2012; 129:e1552-e1561.
6. Fraser A, Nelson SM, Macdonald-Wallis C, Cherry L, Butler E, Sattar N *et al.* Associations of pregnancy complications with calculated cardiovascular disease risk and cardiovascular risk factors in middle age: the Avon Longitudinal Study of Parents and Children. *Circulation* 2012; 125:1367-1380.
7. Berkowitz RS, Goldstein DP. Chorionic tumors. *N Engl J Med* 1996; 335:1740-1748.
8. Matsuo K, Kooshesh S, Dinc M, Sun CC, Kimura T, Baschat AA. Late postpartum eclampsia: report of two cases managed by uterine curettage and review of the literature. *Am J Perinatol* 2007; 24:257-266.
9. Nugent CE, Punch MR, Barr M, Jr., LeBlanc L, Johnson MP, Evans MI. Persistence of partial molar placenta and severe preeclampsia after selective termination in a twin pregnancy. *Obstet Gynecol* 1996; 87:829-831.
10. Raymond D, Peterson E. A critical review of early-onset and late-onset preeclampsia. *Obstet Gynecol Surv* 2011; 66:497-506.
11. Egbor M, Ansari T, Morris N, Green CJ, Sibbons PD. Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. *BJOG* 2006; 113:580-589.
12. Onah HE, Iloabachie GC. Conservative management of early-onset pre-eclampsia and fetomaternal outcome in Nigerians. *J Obstet Gynaecol* 2002; 22:357-362.
13. James JL, Whitley GS, Cartwright JE. Pre-eclampsia: fitting together the placental, immune and cardiovascular pieces. *J Pathol* 2010; 221:363-378.
14. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *BMJ* 2005; 330:565.
15. James JL, Carter AM, Chamley LW. Human placentation from nidation to 5 weeks of gestation. Part I: What do we know about formative placental development following implantation? *Placenta* 2012; 33:327-334.
16. Zhou Y, Damsky CH, Chiu K, Roberts JM, Fisher SJ. Preeclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts. *J Clin Invest* 1993; 91:950-960.

17. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest* 1997; 99:2152-2164.
18. Billington WD. The immunological problem of pregnancy: 50 years with the hope of progress. A tribute to Peter Medawar. *J Reprod Immunol* 2003; 60:1-11.
19. Hunt JS, Petroff MG, McIntire RH, Ober C. HLA-G and immune tolerance in pregnancy. *FASEB J* 2005; 19:681-693.
20. Hunt JS. Stranger in a strange land. *Immunol Rev* 2006; 213:36-47.
21. Guerin LR, Prins JR, Robertson SA. Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? *Hum Reprod Update* 2009; 15:517-535.
22. Le Bouteiller P, Mallet V. HLA-G and pregnancy. *Rev Reprod* 1997; 2:7-13.
23. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B *et al.* Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 1998; 281:1191-1193.
24. Balasch J, Ercilla G, Vanrell JA, Vives J, Gonzalez-Merlo J. Effects of HLA antibodies on pregnancy. *Obstet Gynecol* 1981; 57:444-446.
25. Holmes CH, Simpson KL, Wainwright SD, Tate CG, Houlihan JM, Sawyer IH *et al.* Preferential expression of the complement regulatory protein decay accelerating factor at the fetomaternal interface during human pregnancy. *J Immunol* 1990; 144:3099-3105.
26. Holmes CH, Simpson KL, Okada H, Okada N, Wainwright SD, Purcell DF *et al.* Complement regulatory proteins at the feto-maternal interface during human placental development: distribution of CD59 by comparison with membrane cofactor protein (CD46) and decay accelerating factor (CD55). *Eur J Immunol* 1992; 22:1579-1585.
27. Walport MJ. Complement. First of two parts. *N Engl J Med* 2001; 344:1058-1066.
28. Walport MJ. Complement. Second of two parts. *N Engl J Med* 2001; 344:1140-1144.
29. Flierman R, Daha MR. The clearance of apoptotic cells by complement. *Immunobiology* 2007; 212:363-370.
30. Harboe M, Mollnes TE. The alternative complement pathway revisited. *J Cell Mol Med* 2008; 12:1074-1084.
31. Haas PJ, van Strijp J. Anaphylatoxins: their role in bacterial infection and inflammation. *Immunol Res* 2007; 37:161-175.
32. Klos A, Tenner AJ, Johswich KO, Ager RR, Reis ES, Kohl J. The role of the anaphylatoxins in health and disease. *Mol Immunol* 2009; 46:2753-2766.
33. Cole DS, Morgan BP. Beyond lysis: how complement influences cell fate. *Clin Sci (Lond)* 2003; 104:455-466.
34. Muller-Eberhard HJ. The killer molecule of complement. *J Invest Dermatol* 1985; 85:47s-52s.
35. Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. *Nat Rev Immunol* 2009; 9:729-740.
36. Morgan BP, Meri S. Membrane proteins that protect against complement lysis. *Springer Semin Immunopathol* 1994; 15:369-396.
37. Noris M, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. *Nat Rev Nephrol* 2012; 8:622-33.

38. Fakhouri F, Jablonski M, Lepercq J, Blouin J, Benachi A, Hourmant M *et al.* Factor H, membrane cofactor protein, and factor I mutations in patients with hemolysis, elevated liver enzymes, and low platelet count syndrome. *Blood* 2008; 112:4542-4545.
39. Salmon JE, Heuser C, Triebwasser M, Liszewski MK, Kavanagh D, Roumenina L *et al.* Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. *PLoS Med* 2011; 8:e1001013.
40. Sinha D, Wells M, Faulk WP. Immunological studies of human placentae: complement components in pre-eclamptic chorionic villi. *Clin Exp Immunol* 1984; 56:175-184.
41. Weir PE. Immunofluorescent studies of the uteroplacental arteries in normal pregnancy. *Br J Obstet Gynaecol* 1981; 88:301-307.
42. Wells M, Bennett J, Bulmer JN, Jackson P, Holgate CS. Complement component deposition in uteroplacental (spiral) arteries in normal human pregnancy. *J Reprod Immunol* 1987; 12:125-135.
43. Rampersad R, Barton A, Sadovsky Y, Nelson DM. The C5b-9 membrane attack complex of complement activation localizes to villous trophoblast injury in vivo and modulates human trophoblast function in vitro. *Placenta* 2008; 29:855-861.
44. Tedesco F, Radillo O, Candussi G, Nazzaro A, Mollnes TE, Pecorari D. Immunohistochemical detection of terminal complement complex and S protein in normal and pre-eclamptic placentae. *Clin Exp Immunol* 1990; 80:236-240.
45. Abramson SB, Buyon JP. Activation of the complement pathway: comparison of normal pregnancy, preeclampsia, and systemic lupus erythematosus during pregnancy. *Am J Reprod Immunol* 1992; 28:183-187.
46. Richani K, Soto E, Romero R, Espinoza J, Chaiworapongsa T, Nien JK *et al.* Normal pregnancy is characterized by systemic activation of the complement system. *J Matern Fetal Neonatal Med* 2005; 17:239-245.
47. Derzsy Z, Prohaszka Z, Rigo J, Jr., Fust G, Molvarec A. Activation of the complement system in normal pregnancy and preeclampsia. *Mol Immunol* 2010; 47:1500-1506.
48. Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H. A critical role for murine complement regulator crry in fetomaternal tolerance. *Science* 2000; 287:498-501.
49. Bulla R, Agostinis C, Bossi F, Rizzi L, Debeus A, Tripodo C *et al.* Decidual endothelial cells express surface-bound C1q as a molecular bridge between endovascular trophoblast and decidual endothelium. *Mol Immunol* 2008; 45:2629-2640.
50. Agostinis C, Bulla R, Tripodo C, Gismondi A, Stabile H, Bossi F *et al.* An alternative role of C1q in cell migration and tissue remodeling: contribution to trophoblast invasion and placental development. *J Immunol* 2010; 185:4420-4429.
51. Singh J, Ahmed A, Girardi G. Role of complement component C1q in the onset of preeclampsia in mice. *Hypertension* 2011; 58:716-724.
52. Roberts JM, Hubel CA. The two stage model of preeclampsia: variations on the theme. *Placenta* 2009; 30 Suppl A:S32-S37.

53. Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van AA. A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *Br J Obstet Gynaecol* 1994; 101:669-674.
54. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011; 204:193-201.
55. Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH *et al.* Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. *Am J Pathol* 1997; 151:1809-1818.
56. Plasencia W, Maiz N, Bonino S, Kaihura C, Nicolaides KH. Uterine artery Doppler at 11 + 0 to 13 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2007; 30:742-749.
57. Gomez O, Figueras F, Martinez JM, del RM, Palacio M, Eixarch E *et al.* Sequential changes in uterine artery blood flow pattern between the first and second trimesters of gestation in relation to pregnancy outcome. *Ultrasound Obstet Gynecol* 2006; 28:802-808.
58. Li H, Gudnason H, Olofsson P, Dubiel M, Gudmundsson S. Increased uterine artery vascular impedance is related to adverse outcome of pregnancy but is present in only one-third of late third-trimester pre-eclamptic women. *Ultrasound Obstet Gynecol* 2005; 25:459-463.
59. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol* 1999; 180:499-506.
60. Chesley LC, Cooper DW. Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. *Br J Obstet Gynaecol* 1986; 93:898-908.
61. Cincotta RB, Brennecke SP. Family history of pre-eclampsia as a predictor for pre-eclampsia in primigravidas. *Int J Gynaecol Obstet* 1998; 60:23-27.
62. Nilsson E, Salonen RH, Cnattingius S, Lichtenstein P. The importance of genetic and environmental effects for pre-eclampsia and gestational hypertension: a family study. *BJOG* 2004; 111:200-206.
63. Sutherland A, Cooper DW, Howie PW, Liston WA, MacGillivray I. The indicence of severe pre-eclampsia amongst mothers and mothers-in-law of pre-eclamptics and controls. *Br J Obstet Gynaecol* 1981; 88:785-791.
64. Mogren I, Hogberg U, Winkvist A, Stenlund H. Familial occurrence of preeclampsia. *Epidemiology* 1999; 10:518-522.
65. Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J *et al.* Paternal and maternal components of the predisposition to preeclampsia. *N Engl J Med* 2001; 344:867-872.
66. Skjaerven R, Vatten LJ, Wilcox AJ, Ronning T, Irgens LM, Lie RT. Recurrence of pre-eclampsia across generations: exploring fetal and maternal genetic components in a population based cohort. *BMJ* 2005; 331:877.
67. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet* 2001; 29:306-309.
68. Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. *Hum Reprod Update* 2006; 12:747-755.

69. Heilmann L, Schorsch M, Hahn T, Fareed J. Antiphospholipid syndrome and pre-eclampsia. *Semin Thromb Hemost* 2011; 37:141-145.
70. Cohen D, Berger SP, Steup-Beekman GM, Bloemenkamp KW, Bajema IM. Diagnosis and management of the antiphospholipid syndrome. *BMJ* 2010; 340:c2541.
71. Lynch AM, Murphy JR, Byers T, Gibbs RS, Neville MC, Giclas PC *et al.* Alternative complement pathway activation fragment Bb in early pregnancy as a predictor of preeclampsia. *Am J Obstet Gynecol* 2008; 198:385-389.
72. Dechend R, Muller DN, Wallukat G, Homuth V, Krause M, Dudenhausen J *et al.* Activating auto-antibodies against the AT1 receptor in preeclampsia. *Autoimmun Rev* 2005; 4:61-65.
73. Wang W, Irani RA, Zhang Y, Ramin SM, Blackwell SC, Tao L *et al.* Autoantibody-mediated complement C3a receptor activation contributes to the pathogenesis of preeclampsia. *Hypertension* 2012; 60:712-721.
74. Burton GJ. Oxygen, the Janus gas; its effects on human placental development and function. *J Anat* 2009; 215:27-35.
75. Hung TH, Skepper JN, Burton GJ. In vitro ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. *Am J Pathol* 2001; 159:1031-1043.
76. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP *et al.* Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006; 355:992-1005.
77. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S *et al.* Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003; 111:649-658.
78. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM *et al.* Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006; 12:642-649.
79. Lam C, Lim KH, Karumanchi SA. Circulating angiogenic factors in the pathogenesis and prediction of preeclampsia. *Hypertension* 2005; 46:1077-1085.
80. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005; 308:1592-1594.
81. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN *et al.* A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod* 1998; 59:1540-1548.
82. Redman CW, Sargent IL. Circulating microparticles in normal pregnancy and pre-eclampsia. *Placenta* 2008; 29 Suppl A:S73-S77.
83. Redman CW, Sargent IL. Placental debris, oxidative stress and pre-eclampsia. *Placenta* 2000; 21:597-602.
84. Rajakumar A, Cerdeira AS, Rana S, Zsengeller Z, Edmunds L, Jeyabalan A *et al.* Transcriptionally active syncytial aggregates in the maternal circulation may contribute to circulating soluble fms-like tyrosine kinase 1 in preeclampsia. *Hypertension* 2012; 59:256-264.

85. Smarason AK, Sargent IL, Starkey PM, Redman CW. The effect of placental syncytiotrophoblast microvillous membranes from normal and pre-eclamptic women on the growth of endothelial cells in vitro. *Br J Obstet Gynaecol* 1993; 100:943-949.
86. McCarthy AL, Woolfson RG, Raju SK, Poston L. Abnormal endothelial cell function of resistance arteries from women with preeclampsia. *Am J Obstet Gynecol* 1993; 168:1323-1330.
87. Cockell AP, Poston L. Flow-mediated vasodilatation is enhanced in normal pregnancy but reduced in preeclampsia. *Hypertension* 1997; 30:247-251.
88. Pascoal IF, Lindheimer MD, Nalbantian-Brandt C, Umans JG. Preeclampsia selectively impairs endothelium-dependent relaxation and leads to oscillatory activity in small omental arteries. *J Clin Invest* 1998; 101:464-470.
89. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. *J Clin Invest* 1973; 52:2682-2689.
90. Roberts JM, Edep ME, Goldfien A, Taylor RN. Sera from preeclamptic women specifically activate human umbilical vein endothelial cells in vitro: morphological and biochemical evidence. *Am J Reprod Immunol* 1992; 27:101-108.
91. Roberts JM, Taylor RN, Goldfien A. Clinical and biochemical evidence of endothelial cell dysfunction in the pregnancy syndrome preeclampsia. *Am J Hypertens* 1991; 4:700-708.