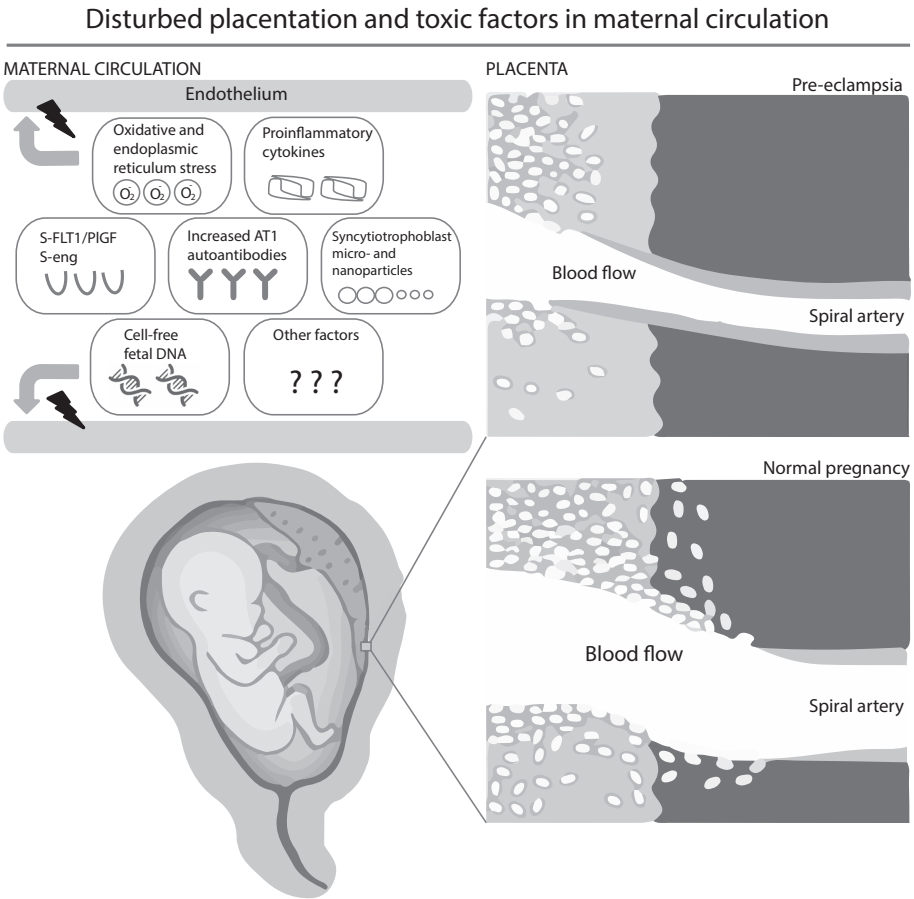
General introduction

1.1 Pre-eclampsia: the 'disease of theories'

Pre-eclampsia (PE) is a pregnancy related disorder, characterized by *de novo* hypertension and proteinuria after 20 weeks of gestation, which occurs in 2-8% of pregnancies worldwide (1-3). The disorder affects multiple organs: the liver, kidneys, clotting system and brain. The placenta, a unique, shared organ of mother and child is almost always affected. The last decades several hypotheses about the pathogenesis of the disorder have been proposed. However, the exact etiology is still unknown. The main hypothesis states that disturbed placental function early in pregnancy leads to the clinical maternal disorder later on in pregnancy (1). During placentation (week 8-18) the remodeling of spiral arteries supplying the uteroplacental circulation fails (1, 4). Placental oxidative and endoplasmic reticulum stress results in the release of toxic factors in the maternal circulation (figure 1). This leads to endothelial dysfunction and the clinical syndrome, in which both mother and fetus are affected (1).

Figure 1.



In general, there seems to be a clear clinical difference between early-onset PE (before week 34) and late-onset PE (after week 34). Early-onset PE tends to be more severe and more associated with the placental pathology. Late-onset PE is more common than early-onset PE and seems to have a milder presentation. However, late-onset PE can still lead to severe maternal and foetal pathology, like eclampsia, especially in low-resource settings (5, 6). The heterogeneous character of PE makes it complicated to unravel the exact pathophysiology and also to develop a predictive screening test. In the last decades many researchers have been looking for a screening test with a good predictive value to make it possible to estimate the risk of PE of individual pregnant women. This will lead to personalized care tailored to the individual: interventions and preventive therapies can be applied to improve care and reduce maternal and foetal morbidity and mortality.

1.2 Brain involvement in PE

From a historical perspective, the name PE is derived from the process prior to the onset of one of the most severe complications: eclampsia. Already in ancient times the Greek wrote: "*In pregnancy, drowsiness and headache accompanied by heaviness and convulsions is generally bad*". In 1849 Dr. William Tyler Smith proposed that cerebral congestion during pregnancy was the result of a state of increased fullness of the circulation. One of his theories about the cause of the disorder was that toxic elements play a role and failure to waste these elements resulted in toxemia of pregnancy, causing irritation to the nervous center (7).

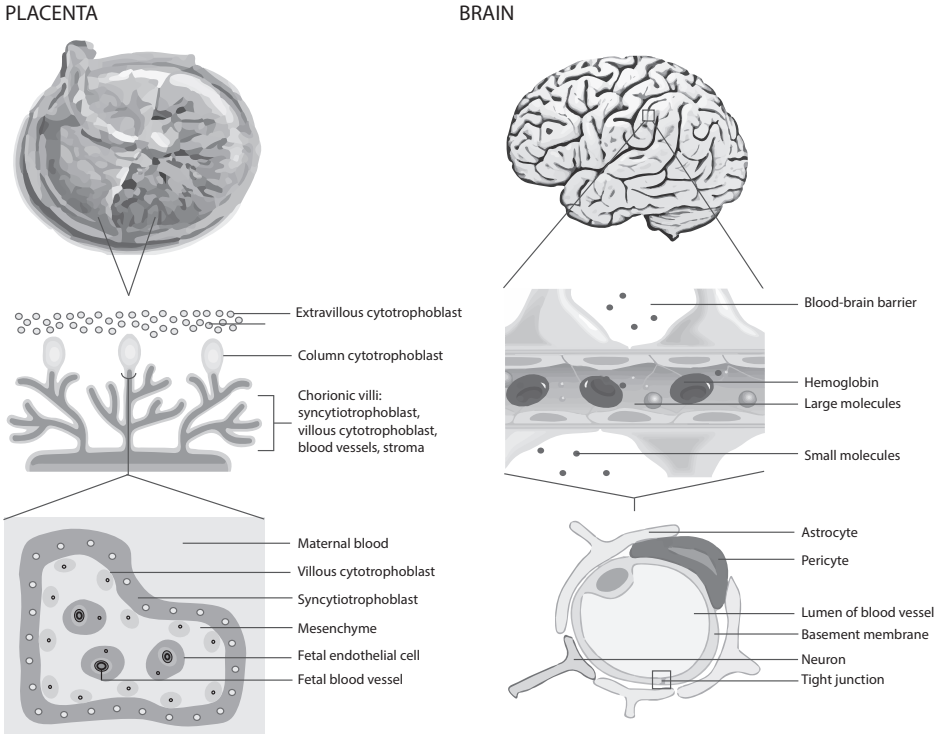
Nowadays, the exact origin of brain pathology in pre-eclampsia still isn't elucidated. The current idea is that a disturbed auto-regulatory response of the brain leads to increased blood pressure and endothelial cell dysfunction, which clinically presents as posterior reversible encephalopathy syndrome (PRES) (8-10). Although this pathogenesis is not yet fully understood, the blood-brain barrier (BBB) may play an important role in this process (11). Endothelial cells of the BBB differ from those in peripheral tissues, by having a low rate of endocytosis and being coupled by tight junctions to restrict the amount of para-cellular fluid (12). These functions ensure that not all blood constituents can pass freely into the extracellular space in the central nervous system (figure 2).

Our hypothesis is that during (severe) PE disruption of the BBB may be caused by the same pathophysiology as endothelial damage in other highly vascularized organs during PE, like the placenta, kidney and liver (figure 1 and 2).

1.3 Aim of the thesis

1. To investigate *laboratory biomarkers* of pre-eclampsia, using proteomics and epigenetics techniques.
2. To investigate *brain involvement* during pregnancy and pre-eclampsia, using proteomics and neurophysiological techniques.

Figure 2. Placenta and brain: two highly vascularized organs with a barrier function

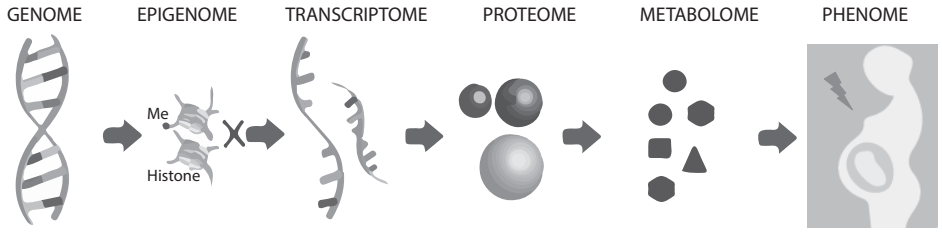


In the following paragraphs three different techniques to achieve these aims are described:

1.3.1 Proteomics: a hypothesis-driven and hypothesis-generating approach

Before the onset of systemic maternal disease, several toxic factors are released in the maternal circulation. These circulating factors may involve oxidative stress factors, inflammatory factors and vasoactive factors (figure 1). Many of those are proposed as potential biomarkers for PE. The last decades different 'omic' techniques have been helpful in finding new biomarkers and understanding more about the etiology of several disorders (figure 3). Proteomics is a promising technique in biomarker research. In a sensitive and specific way the abundance of proteins can be measured by using mass-spectrometry. This technique is able to explore and to quantify the most downstream markers of physiologic and pathologic processes in the human body. Targeted and exploratory proteomics techniques can respectively validate candidate protein biomarkers and generate new hypotheses. Especially in a complicated disorder like PE this technique might be helpful (13). In this thesis we present a *targeted* proteomics study in which we quantify two candidate biomarkers for PE (calcyclin and heat shock protein 90 (HSP90)) and an *exploratory* study in which we reveal the cerebrospinal fluid proteome of women with severe PE.

Figure 3. 'Omics' techniques may be helpful in unravelling the origin of several diseases



Research questions:

- Which laboratory biomarkers for PE are described in the literature and what is their predictive value? *(chapter 2)*
- Do the serum proteins calcyclin and HSP90 have predictive value as biomarkers for PE? *(chapter 3)*
- Is the cerebrospinal fluid (CSF) protein profile different in patients with (severe) PE compared to normotensive pregnant controls? *(chapter 6)*

1.3.2 Epigenetics: pregnancy as a window to future health

Since the Development Origin of Health and Disease (DOHaD) paradigm has been introduced, epigenetic research has proven its value in this field. Suboptimal placental growth and function is associated with morbidity in the perinatal period as well as an increased risk of cardiovascular disease in childhood and adult age. DNA methylation is one of the major epigenetic mechanisms that may alter gene expression. Nutrient and environmental exposures influence DNA methylation during pregnancy and thus may also affect offspring growth and development (14-16). Especially in a pregnancy disorder, like PE, which is associated with placental pathology, altered DNA methylation may play an important role. This alteration may also affect the future generation. Therefore we aim to measure DNA methylation of two specific candidate gene pathways in placental tissue, umbilical cord and human umbilical vein endothelial cells (HUVECs). These gene pathways are the circadian clock related pathway and the 25 genes encoding for the proteins we found in the exploratory proteomics study *(chapter 7)*.

Research questions:

- Is DNA methylation of circadian clock (related) genes different in placental tissue, umbilical cord white blood cells and HUVECs of patients with PE compared to uncomplicated controls and pregnancies complicated by preterm birth (PTB) and foetal growth restriction (FGR)? *(chapter 4)*
- Is DNA methylation of 25 candidate genes for pre-eclampsia different in placental tissue, umbilical cord white blood cells and HUVECs of patients with PE compared to

uncomplicated controls and pregnancies complicated by preterm birth (PTB) and foetal growth restriction (FGR)? *(chapter 5)*

1.3.3 Neurophysiology: Visual Evoked Potentials (VEP)

Different neuroimaging and neurophysiological studies show abnormalities during PE (17). However, currently it is not usual in standard care for patients with (severe) PE to evaluate brain function by using laboratory or neurophysiological tests. Studying neurophysiological parameters is helpful in understanding the adaptation of the brain to pregnancy and hypertensive disorders. Visual symptoms are present in approximately 25% of women with PE. The functional status of the visual cortex during PE or hypertension in pregnancy that could lead to visual symptoms has not been investigated (18). The origin of these visual disturbances is likely to be localized in either the neurological or ophthalmic pathways (18). The objective of our study was to test neurophysiological function during pregnancy and postpartum by means of VEPs. VEPs measure the functional integrity of the visual pathway from retina to the occipital cortex of the brain (19). Abnormal VEPs may present as changes in latency, amplitude, topography and waveform.

Research question:

- Do women with a hypertensive disorder of pregnancy have altered VEPs compared to women with a normotensive pregnancy? (*chapter 7*)