General introduction, aims and outline

GENERAL INTRODUCTION

Preeclampsia (PE) is a severe placenta-related pregnancy disorder, that complicates around 5-8% of pregnancies.[1] PE can have detrimental consequences during pregnancy for the mother, as it increases the risk of for example stroke, liver rupture, and lung edema. It also contributes significantly to perinatal morbidity, as approximately 12% of PE cases is complicated by fetal growth restriction, and around 20% results in preterm birth.[2] Besides these short term consequences, PE is also associated with health risks later in life for both mother and child. Women who have suffered from PE have a 2-4 times higher risk of developing cardiovascular diseases.[3, 4] Furthermore, prematurity and low birth weight increase the risk of cardiopulmonary and neurological impairment in children.[5, 6]

Nowadays PE is defined as de novo hypertension after the 20th week of gestation, along with evidence of maternal organ damage and/or fetal growth restriction.[7] However, as early as 400 BC, Hippocrates already wrote that 'a headache accompanied by heaviness and convulsions during pregnancy is considered bad'.[8] In that time it was thought that all female diseases resulted from an imbalance in body fluids, and therefore treatments existed of dietary changes, purging and blood-letting.[9] In the 17th century, the word 'eclampsia' arose and the disease was systematically described for the first time. In an attempt to prevent convulsions, phlebotomies during pregnancy were recommended.[10] In the 19th century, the disease was called 'toxemia', as it was thought that it resulted from the inability to eliminate an increase in waste products.[11] Treatment still mainly existed of bleeding and purging, in order to eliminate the excess of toxic elements. It was not until the 20th century that the pathophysiology of PE was linked to the abnormal placentation in early pregnancy.[12] Although still not fully elucidated, it is now known that increased placental vascular resistance leads to an imbalance in pro - and antiangiogenic factors, causing generalized endothelial dysfunction.[13, 14] The latter is characterized by disturbances in different vascular pathways, e.g. decreased activity of the nitric oxide pathway and increased activity of the endothelin system.[15, 16] Furthermore, there is an imbalance in the immune system response, with a shift towards pro-inflammatory conditions.[17, 18]

To date, still no cure is available for PE, except for delivery of the placenta. Patients are often stabilized with antihypertensive drugs, such as methyldopa and/or calcium antagonists, and magnesium sulfate to prevent further complications and prolong pregnancy as long as possible. Targeting the vascular endothelial dysfunction in PE is a promising strategy in developing new therapeutic options.

The *ex vivo* placental perfusion system

For the research described in this thesis we used dual-sided *ex vivo* placental perfusion of isolated cotyledons, an established experimental model to study the transfer of drugs across the human placenta, their subsequent effects and placental metabolism. It is the only reliable method to predict *in vivo* fetal exposure to maternally administered compounds without imposing risks on mother or child.[19, 20] The model was first described in 1967 by Panigel *et al.*[21] and later modified by Schneider *et al.* in 1972.[22] The model used for the studies in this thesis has been adapted from the model described by Schalkwijk *et al.*[23] It consists of a plastic perfusion chamber and two peristaltic roller pumps. Heating devices and a water bath keep the temperature in the setup at 37 °C. Maternal and fetal perfusion media are aerated continuously with 95% O_2 – 5% CO_2. After arrival at the lab directly after birth, an intact cotyledon is selected and the corresponding chorionic artery and vein pair is cannulated to establish the fetal circulation. Subsequently, the cotyledon is cut from the rest of the placenta and placed inside the perfusion chamber. Maternal circulation is created by placing four blunt cannulas in the intervillous space and outflow from the intervillous space is collected in a reservoir. Changes in fetoplacental pressure and pH are recorded using acquisition software. A schematic overview of our placental perfusion model is shown in Figure 1.

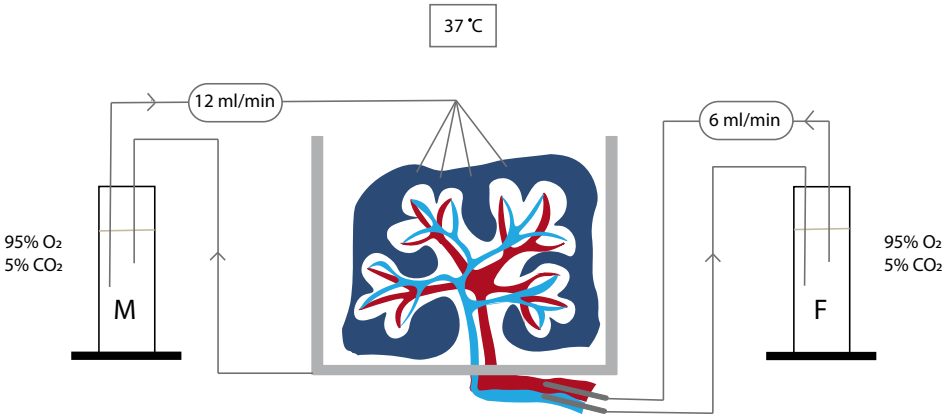

Figure 1. Schematic overview of the placental perfusion model in our lab.

AIMS AND OUTLINE OF THE THESIS

In this thesis new treatment strategies for PE are discussed, targeting the placental vascular dysfunction that plays a key role in the pathogenesis of this disease. By using *ex vivo* techniques, such as perfusion of the isolated cotyledon and wire-myography, the human placenta is studied without the risk of harming either mother or fetus.

The aims of this thesis are:

1. To understand placental vascular reactivity in health and disease
2. To evaluate possible new treatment options for PE, using *ex vivo* placental perfusion

Chapter 2 provides an extensive overview of the vascular reactivity profile of the human placenta. It summarizes the most important pathways and factors that are involved in the regulation of (placental) vascular function during healthy pregnancy and changes associated with PE. Furthermore, potential treatment strategies interfering with these changes are discussed. **Chapters 3-6** focus on experimental therapeutic strategies targeting the vascular dysfunction seen in PE. In **Chapter 3** we study the transfer and effect of the phosphodiesterase-5 inhibitor sildenafil in placentas from healthy and PE pregnancies. In **Chapter 4** we try to elucidate the vasodilator mechanism of pentoxifylline by studying its effect in porcine coronary arteries and human chorionic plate arteries. **Chapter 5** discusses the possibility of using endothelin receptor antagonists for PE treatment. It includes an overview of all cases reported in literature of women who were exposed to these drugs during pregnancy. Following this, in **Chapter 6** we examine the transfer and effects of the endothelin receptor antagonists macitentan, sitaxentan and ambrisentan in the human placenta. **Chapter 7** explores the associations between first-trimester *in vivo* placental vascular parameters and *ex vivo* placental vascular function at term. **Chapter 8** provides a general discussion and suggestions for future research, and in **Chapter 9** this thesis is summarized.

REFERENCES

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010;376:631-644
2. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009;33:130-137
3. Barker DJ. The origins of the developmental origins theory. J Intern Med. 2007;261:412-417
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. Jayet PY, Rimoldi SF, Stuber T, Salmon CS, Hutter D, Rexhaj E, Thalmann S, Schwab M, Turini P, Sartori-Cucchia C, Nicod P, Villena M, Allemann Y, Scherrer U, Sartori C. Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia. Circulation. 2010;122:488-494
6. Hintz SR, Kendrick DE, Wilson-Costello DE, Das A, Bell EF, Vohr BR, Higgins RD, Network NNR. Early-childhood neurodevelopmental outcomes are not improving for infants born at <25 weeks' gestational age. Pediatrics. 2011;127:62-70
7. American College of Obstetricians and Gynecologists Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the american college of obstetricians and gynecologists' task force on hypertension in pregnancy. Obstet Gynecol. 2013;122:1122-1131
8. Chadwick J, Mann WN. Hippocrates. The medical works of hippocrates. England: Blackwell Scientific Publications; 1950.
9. Green MH. The transmission of ancient theories of female physiology and disease through the early middle ages. 1985
10. Chesley LC. History and epidemiology of preeclampsia-eclampsia. Clin Obstet Gynecol. 1984;27:801-820
11. Smith WT. Parturition and the principles and practice of obstetrics. Philadelphia: Lea & Blanchard; 1849.
12. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of pre-eclampsia. Obstet Gynecol Annu. 1972;1:177-191
13. Roberts JM. Pathophysiology of ischemic placental disease. Semin Perinatol. 2014;38:139-145
14. Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. Annu Rev Med. 2008;59:61-78
15. Osol G, Ko NL, Mandala M. Altered endothelial nitric oxide signaling as a paradigm for maternal vascular maladaptation in preeclampsia. Curr Hypertens Rep. 2017;19:82
16. Granger JP, Spradley FT, Bakrania BA. The endothelin system: A critical player in the pathophysiology of preeclampsia. Curr Hypertens Rep. 2018;20:32
17. Azizieh F, Raghupathy R, Makhseed M. Maternal cytokine production patterns in women with pre-eclampsia. Am J Reprod Immunol. 2005;54:30-37
18. Jonsson Y, Ruber M, Matthiesen L, Berg G, Nieminen K, Sharma S, Ernerudh J, Ekerfelt C. Cytokine mapping of sera from women with preeclampsia and normal pregnancies. J Reprod Immunol. 2006;70:83-91
19. Hutson JR, Garcia-Bournissen F, Davis A, Koren G. The human placental perfusion model: A systematic review and development of a model to predict in vivo transfer of therapeutic drugs. Clin Pharmacol Ther. 2011;90:67-76
20. Conings S, Amant F, Annaert P, Van Calsteren K. Integration and validation of the ex vivo human placenta perfusion model. J Pharmacol Toxicol Methods. 2017;88:25-31

21. Panigel M, Pascaud M, Brun JL. Radioangiographic study of circulation in the villi and intervillous space of isolated human placental cotyledon kept viable by perfusion. Journal of Physiology. 1967;59:277
22. Schneider H, Panigel M, Dancis J. Transfer across the perfused human placenta of antipyrine, sodium and leucine. Am J Obstet Gynecol. 1972;114:822-828
23. Schalkwijk S, Greupink R, Colbers AP, Wouterse AC, Verweij VG, van Drongelen J, Teulen M, van den Oetelaar D, Burger DM, Russel FG. Placental transfer of the hiv integrase inhibitor dolutegravir in an ex vivo human cotyledon perfusion model. J Antimicrob Chemother. 2016;71:480-483