Placental hypoxia

Valorization

This chapter addresses the relevance and contribution of this thesis to society. The general aim of this project was to investigate the effect of preeclampsia (PE) on placental histological villous maturation and to set up a model for PE to examine the contribution of mitochondria in the generation of placental oxidative stress, its effect on the placental secretome and its subsequent role in acute and sustained vascular contractility, morphology, and endothelial integrity.

Valorization is the term used for the process of value-creation out of knowledge, by making this knowledge suitable and available for economic or societal utilization and to translate this into high-potential products, services, processes, or industrial activity.

First, in this valorization chapter, the current epidemiological data that describes the health status of adults and the economic burden of PE will be presented. Secondly, potential of our results for

- better characterization of the disease subtype to improve diagnosis and subse quent treatment.
- the prevention of PE associated with nutritional interventions via the consump tion of identified diets.

Social and economic impact of PE and relevance of this study

There are half a million maternal pregnancy-related deaths worldwide of which 1% occurs in high-income countries (1). PE is an enigmatic and complex hypertensive disorder affecting seemingly healthy pregnant women and carries substantial health risks to both mother and baby. Despite significant research, PE continues to affect 10 million pregnant women and kills 76,000 mothers and 500,000 babies per year worldwide (2). A large regional integrated health care system study in Pennsylvania, US used electronic health records and billing data to identify mother-singleton infant pairs. The final study population included 712 matched mother-infant pairs comparing the medical costs related to uncomplicated pregnancies versus PE-complicated pregnancies. The mean combined maternal and infant medical cost of $41,790 were significantly higher than those for the uncomplicated cohort of $13,187 and was largely driven by the infant care cost, which was dominated by neonatal intensive care unit costs, which was almost 7 times higher compared to the control group (3). As PE is the leading cause of maternal mortality in the Netherlands, the prevalence of PE is comparable and high income and middle to low-income countries show similar proportions of maternal deaths related to PE, similar costs are expected in the Nederland's (4). The broad clinical picture of this complication is the reason for a delayed diagnosis, increasing the risk for complications for mother and child and

it affects maternal health and child's health and wellbeing, especially if the child is born prematurely. PE is a commonly sudden and a distressing life experience, particularly if women felt severely ill, gave birth too early, or if they lost their baby. With secondary neonatal intensive care necessity, PE strongly links to a post-traumatic stress disorder, and depression and women often find difficulties to reintegrate into society after giving birth further demonstrating the broad economic burden of PE.

PE causes not only short but also long term consequences related to future metabolic and cardiovascular events for the mother and child (5). A delay in diagnosis and access to appropriate care is a core cause of PE-related severe morbidity and mortality worldwide and is the main reason for the lack of curative options besides preterm delivery of the fetus. Malformation and subsequent impaired function of the placenta during PE play a pivotal role in the development of suboptimal cardiovascular adaptations during pregnancy leading to a hypertensive-complicated pregnancy. During the disease state, however, there are currently no curative treatment options available, but only symptomatic treatment to correct the underlying cardiovascular shortcomings, which includes optimizing blood-pressure and -flow and protection from possible imminent seizures. Better understanding of the abnormal adaptation processes of the placenta and the maternal vascular system during a PE-complicated pregnancy may result in better diagnostic tools, subsequent shortening of diagnosis, access to appropriate care, and the selection of more targeted therapies. Although several risk factors have been identified, it is difficult to develop effective strategies for the prevention and treatment of PE (6). Strategies applied nowadays are diverse and include antenatal surveillance, modification of lifestyle, dietary interventions, and symptomatic directed pharmacological therapy such as antihypertensive.

Until now, characterization of the two existing subclasses (early and late-onset PE) is only based on the gestational age at diagnosis. Some investigators have considered that the two subtypes of PE represent more than one pathophysiologic process (7, 8). This variety of separate pathological entities are yet to be defined and might be an explanation for the failing population-based interventions in women at high risk for developing PE. Allocating all cases to one general PE group may explain why, despite the strong association of PE with oxidative stress, human studies using systematically acting antioxidants like vitamin C and E as a treatment for PE were generally unsuccessful (9). The Barker hypothesis suggests that nutritional exposure in utero has effects that even after birth may last a lifetime. Moreover, that gestational age, which is generally shortened by PE is predictive of heart disease in the offspring later in life, decades post-partum affecting maternal, foetal and newborn health (3). In addition to these potential long-term effects of PE, maternal diet and nutritional

status are known to have more immediate effects on maternal, fetal, and newborn health. Over the past 20 years, the importance of preventive nutrition in enhancing pregnancy outcomes has been a very valuable area of research tailoring at a reduction of major birth defects, prolonging pregnancy to term, and avoiding maternal complications. When these complications could be avoided, it not only results in cost savings but also in a reduction in linked emotional and psychological costs. At present, there is still a major opportunity in optimizing pregnancy outcomes of preterm birth-related pregnancy complications like PE by implementing essential micronutrients such as vitamin C and E in the diet. Deficiencies in essential micronutrients have profound effects on the physical as well as the maternal capabilities of the offspring and significantly promotes maternal morbidity and mortality. A study by Chappell et al., included 283 pregnant women at risk for PE in a placebo-controlled trial and daily supplementation of vitamin C (1,000 mg) and vitamin E (400IU) resulted in a 61% reduction in developing PE. However, other studies using a similar dose of vitamin C and E could not replicate the Chappell findings. Inconsistencies in the findings of these and similar studies suggest heterogeneity in population groups, different pre-existing conditions, and other factors independent of the basic research (10-12). The failing identification of PE subgroups based on its clinical presentation and its following limitations in current PE research underlines the need for a better understanding of the molecular mechanisms involved in the development of placental oxidative stress and subsequent alterations in its secretome, which drives the progression of PE. Improved knowledge as presented in this thesis presenting these fundamental mechanisms of PE pathogenesis will provide researchers with a tool to allocate patients to specific PE subgroups, and will help in the formation of homogenous PE test groups and will create better awareness of the diverse pathological image of PE.

As placental maldevelopment and failing adaptation are central to the pathogenesis of PE, in the first research chapter (**Chapter 2**), we identified 11 parameters of histological villous remodeling, which demonstrate accelerated villous maturation in PE placentae. These identified parameters as presented in our study, not only allow us to study histological adaptations that take place in the placenta complicated with PE, but may also be used to correlate with clinical parameters of the mother and subsequently help in the identification of different clinical subtypes of PE. Advancing our understanding of the pathophysiology underlying PE through the identification of clinically relevant disease subtypes would not only clarify the heterogeneity observed in this disorder but will also help in the development and/or application of etiology-focused screening tools and therapies for PE. These tools would direct the care of women and their offspring affected by PE towards an etiological-based inter-

vention, which will expand the current symptomatic-focused intervention.

Although mitochondria are forming the major source for ROS and are central to the regulation of cellular metabolism, redox state, and cell fate, few studies have examined the connection between impaired oxygen supply to the placenta and mitochondrial dysfunction and ROS. In our study (**Chapter 3 and 4**) we now for the first time, comprehensively demonstrate increased oxidative stress, which mainly originates from mitochondria, decreased mitochondrial content, and signs for reduced mitochondrial biogenesis, and increased mitophagy and mitochondrial fission in placentae complicated with PE or after exposure to hypoxia. Our findings imply that mitochondrially targeted antioxidant-based intervention aimed at preventing mitochondrial dysfunction and excessive ROS formation may have therapeutic potential in pregnancy complications like PE.

The specifically identified alterations related to mitochondrial function and oxidative stress may together with the histological adaptations in the placenta found in **chapter 2**, be linked to the clinical profile of the mother and serve as a tool to efficiently identify more subgroups of PE in larger cohorts. Moving forward, the use of a PE subtype paradigm may allow for more homogeneous patient population selection in study designs, leading to greater scientific contributions in the understanding of PE pathophysiology, the discovery of highly accurate predictive biomarkers, and targeted therapeutic interventions tailored to each disease subtype.

Placental ischemia/hypoxia leading to oxidative stress (13) results in the production of a variety of placental secreted factors (secretome) that collectively have profound effects on blood flow and arterial pressure regulation. Besides predisposing women to PE, also hypertension, microalbuminuria, dyslipidemia, a pro-inflammatory phenotype, obesity, and insulin resistance are observed in higher numbers in women in later life after a PE-complicated pregnancy (14). In this thesis, we show for the first time that placental oxidative stress upon placental hypoxia triggers the placental secretome resulting in vascular contraction mediated via the AT1 and ET-1 receptors, and increases endothelial permeability. Increased vascular responsiveness to contractile compounds like TXA2 was associated with increased vascular proliferation and subsequent increased arterial thickness of the media. A better understanding of the complex interplay between the stressed placenta in PE and the maternal cardiovascular system may help in the identification of new serum biomarkers for the prediction of PE and in the design of new diagnostic approaches for better clinical management. It will also improve our knowledge of the long-lasting maternal vascular consequences opening new postpartum treatment and follow-up strategies.

In conclusion, this thesis provides new insights into the etiology and progression of PE. New diagnostic approaches for better clinical management and early prevention or amelioration of the progression of the disease, will not only positively influence morbidity and mortality rates during pregnancy, but also reduce the risk of maternal and foetal complications in later life. Moreover, in this thesis we improved knowledge on specific histological alterations found in the PE-placenta, comprehensively demonstrated mitochondrial impairments, and following oxidative stress-related alterations in the placental secretome, and its subsequent effect on the vascular system. This knowledge not only provides researchers with a tool that will help in identifying specific PE subgroups but also opens new perspectives in new efficient targeted therapeutic interventions tailored to each disease subtype and well-directed postpartum treatment and follow-up strategies.

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