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## Oxidative stress in placental pathology

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### ABSTRACT

The most important function of the placenta is the exchange of nutrients and oxygen between a mother and her fetus. To establish a healthy functioning placenta, placentation needs to occur with adequate remodelling of spiral arteries by extravillous trophoblasts. When this process is impaired, the resulting suboptimal and inadequate placenta function results in the manifestation of pregnancy complications. Impaired placenta function can cause preeclampsia and leads to fetal growth restriction due to hypoxia. Presence of hypoxia leads to oxidative stress due to an imbalance between reactive oxygen species and antioxidants, thereby causing damage to proteins, lipids and DNA. In the placenta, signs of morphological adaptation in response to hypoxia can be found. Different placental lesions like maternal or fetal vascular malperfusion or chronic villitis lead to a decreased exchange of oxygen between the mother and the fetus. Clinically, several biomarkers indicative for oxidative stress, e.g. malondialdehyde and reduced levels of free thiols are found. This review aims to give an overview of the causes and (potential) role of placental oxidative stress in the development of placental parenchymal pathology and its clinical consequences. Also, therapeutic options aiming at prevention or treatment of hypoxia of the placenta and fetus are described.

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### 1. Introduction

In human pregnancy, the placenta fulfills the role of exchanger of nutrients and oxygen between the mother and the fetus. This exchange takes place at the interface of the placental villi with their vasculosyncytial membranes (VSM) and the intervillous space in which the maternal blood flows [1]. This process enables the fetus to grow and develop normally. When placental function is compromised, fetal growth is affected resulting in fetal growth restriction (FGR).

To establish a healthy placental function, the fertilized oocyte needs to be implanted properly, followed by a coordinated invasion of extravillous trophoblasts into the maternal decidua and spiral arteries resulting in remodelling of spiral arteries and a low resistance circulation in the intervillous space [2]. When invasion of extravillous trophoblast is impaired, placental function is

consequently diminished. Although many types of placental lesions and maternal conditions can lead to pregnancy complications and poor fetal outcome, most research has focused on the etiology and treatment of preeclampsia (PE), a syndrome associated with maternal endothelial cell dysfunction, proteinuria and hypertension; and on FGR [3]. The exact pathophysiological pathways underlying these diseases are not fully understood, although several pathways have been suggested [4]. The incidence of PE has been described to lie between 2% and 15% in all pregnancies and can result in serious fetal and or maternal morbidity and mortality [5–7].

PE results in around 15% preterm births [8], primarily because of an indicated delivery. In the mother, PE can evolve into eclampsia with hypertension and seizures. It can also lead to persistent dysregulation of systemic physiology after pregnancy [9]. Short-term complications of PE for the fetus are FGR and fetal demise, especially in early onset PE [10]. FGR is defined as a fetus who does not reach its genetic growth potential and affects up to 15% of all pregnancies [3,11,12]. Long-term complications are adult diseases which have their origin during fetal development (also known as the Barker hypothesis) such as cardiovascular diseases [13], neurodevelopmental disorders [14] and metabolic syndrome [15]

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including diabetes mellitus [16].

In PE, histological changes of the placental parenchyma are seen as a result of ischemia induced by for example maternal vascular malperfusion (like decidual arteriopathy, abruption and infarction). Several similar but also different types of placental lesions are seen in FGR, for example ischemic changes in maternal vascular malperfusion; thrombosis and avascular villi in fetal vascular malperfusion, and a lymphocytic intervillous infiltrate in chronic villitis [11,17].

In placental ischemia the balance between reactive oxygen species (ROS) like superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) and antioxidants is disrupted, leading to oxidative stress causing damage to proteins, lipids and DNA [18]. Oxidative stress is characterized by formation of large amounts of ROS in among others cell membranes, endoplasmic reticulum and mitochondria [19]. The placenta produces various factors [3], such as the antioxidants, including the vasodilator nitric oxide (NO), that are all excreted into the maternal circulation under physiological conditions. Their aberrant expression however is associated with several pregnancy complications [20]. In addition to its vasodilatory actions the role of the gasotransmitter NO in complicated pregnancy is also to scavenge oxidants thereby decreasing cellular and tissue damage. Systemic presence of gasotransmitters (NO,  $H_2S$  (hydrogen sulfide) and CO (carbon monoxide)) and the blood levels of other antioxidant substances such as free thiols [21,22] could serve as biomarkers to detect (early) PE and/or FGR. Additionally, gasotransmitters could potentially be used as target for intervention to treat or ameliorate processes involved in the development of placental disease. Additional measures for oxidative stress include the amount of malondialdehyde in maternal blood, a marker of lipid peroxidation [7]. Another form of oxidative stress results from ischemia/reoxygenation (i.e. ischemia/reperfusion) injury, as seen after intermittent placental perfusion due to periods of vasoconstriction in cultured villous samples from term human placenta [23].

Since the pathophysiology of PE is multifactorial and not fully understood yet, it has been difficult to develop therapeutic or preventive strategies. At the moment, the only option to treat PE is to deliver the placenta.

The aim of this review is to give an overview of the causes and the (potential) role of placental oxidative stress in the development of placental parenchymal pathology and its clinical consequences. Also, therapeutic options aiming at prevention or treatment of hypoxia of the placenta and fetus are discussed.

## 2. Placentation

### 2.1. Normal placentation and function of the first trimester placenta

After fertilization the blastocyst implants in the maternal decidua and the trophoblast superficially invades the myometrium. Until nine weeks post-menstruation, the complete gestational sac is covered with placental villi. At the end of the first trimester, when the embryo is between 31 and 60 mm crown-rump length, the villi at the implantation site form the placenta and the opposed villi regress and form the placental chorionic membrane [24].

In early pregnancy, maternal blood flow is not yet established in the intervillous space of the placenta. Maternal spiral artery remodelling starts directly after implantation of the blastocyst with invasion of extravillous trophoblast (EVT) cells into the decidua and formation of a continuous EVT shell at the maternoplacental interface [25]. A subset of these EVT cells will become endovascular trophoblastic cells transforming the spiral arteries into dilated vessels arising from the muscular layer, with wide diameter. However, these wide diameter vessels are plugged by EVT in the

first trimester, thereby restricting flow of oxygenated blood in the developing placenta. This results in low oxygen tension in the intervillous space, which is essential for normal embryogenesis and organogenesis as the developing fetus is lacking mechanisms, especially in the mitochondria, to prevent free radical damage [18]. Transfer of nutrients from the mother to the developing fetus is in this stage mediated via diffusion.

When embryogenesis is completed, the EVT plugs begin to dissolve thereby establishing a continuous low flow perfusion of oxygenated blood into the placental intervillous space [25,26] (Fig. 1B, left half, "normal"). The exact mechanism by which EVT plugs dissolve remains unclear [27]. This process starts at the peripheral margin of the placenta expanding to the center of the placenta, producing a gradual increase in maternal blood flow and thus oxygen tension. Invasion of EVT in the spiral arteries is deeper in the central region of the placenta compared to the periphery; therefore it takes longer to dissolve the plugs and this explains the gradual increase in oxygen tension [27]. This phenomenon results in a shift from low oxygen tension to a higher oxygen tension in the intervillous space at the end of the first trimester [25,26].

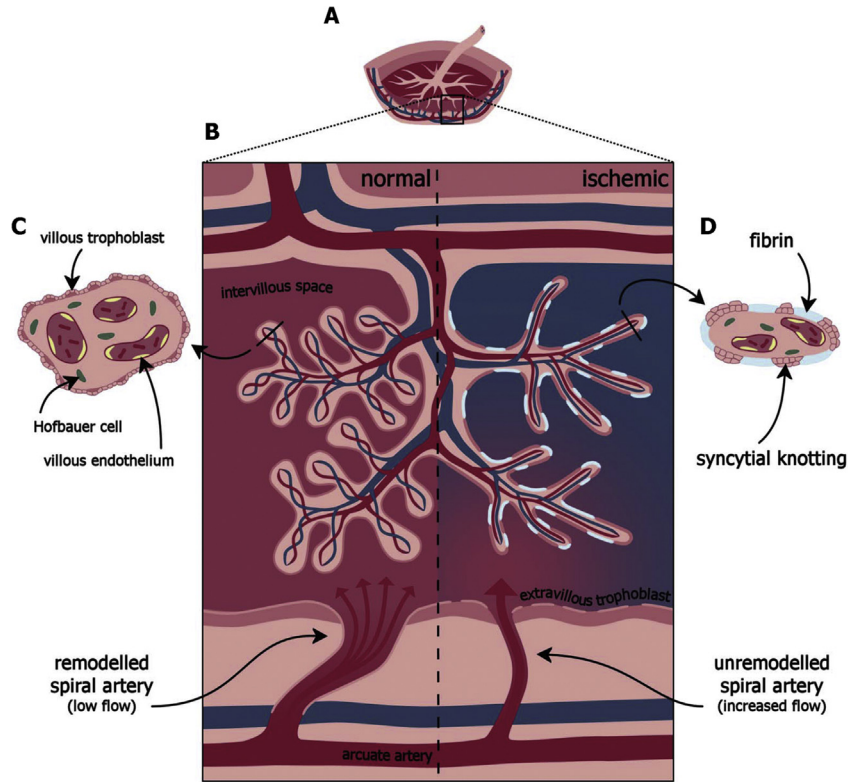
From the second trimester onwards, the placental villi are composed of Hofbauer cells (macrophages), stromal fibroblasts and fetal capillaries, covered with a basal membrane and a bilayer of villous trophoblast (Figs. 1C and 2). The inner layer of this bilayer is composed of cytotrophoblast, the outer layer of syncytiotrophoblast. When villi are fully developed, the fetal capillary walls make intimate contact with the villous outer surface, and vasculosyncytial membranes (VCM) are formed. At these VCM the majority of gas exchange between fetus and mother takes place. To establish the formation of membranes, the syncytiotrophoblasts cluster together to form syncytial knots thereby decreasing the distance for gas exchange (Fig. 2) [28].

### 2.2. Abnormal placentation

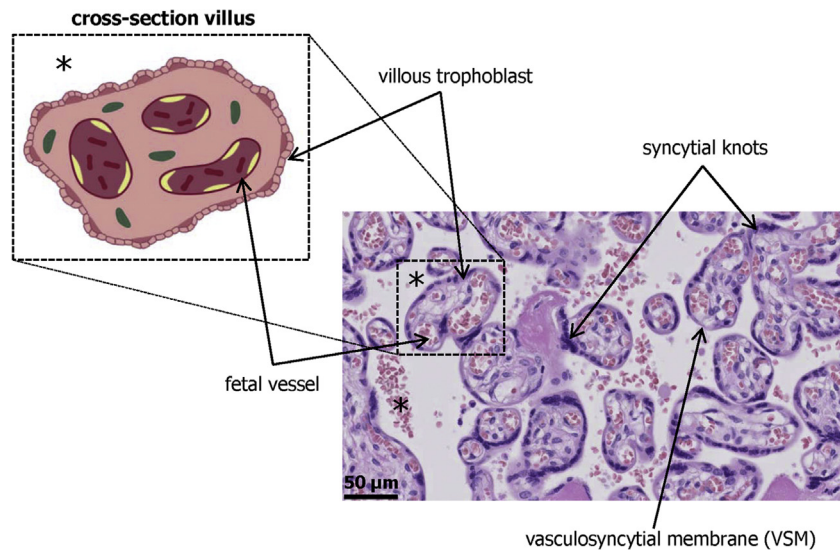
When the EVT cells fail to invade the spiral arteries, incomplete plugging and fragmentation of the EVT shell occurs. This leads to premature onset of maternal placental circulation, and consequently to a premature increase in oxygen tension that successively results in a relative hyperoxic environment. Besides a rise in oxygen tension, the complete intervillous space of the placenta is perfused with maternal blood instead of the gradual process beginning at the peripheral margin of the placenta [29]. Increase in oxygen tension could lead to formation of reactive oxygen species (ROS) for example through increased formation of superoxide radicals reflected by an increase in anti-nitrotyrosine in miscarriage samples [19]. The mean oxygen tension measured within the fetoplacental unit changes from <20 mmHg at 8–10 weeks menstrual age to about 60 mmHg at 12–13 weeks [30] in uncomplicated pregnancy, which is still lower than the oxygen tension of the air we breathe. Normally, in the low oxygen environment within the intervillous space of first trimester placenta, syncytiotrophoblast is not exposed to ROS. Syncytiotrophoblast is in particular sensitive to ROS because this layer lacks sufficient concentrations of antioxidative enzymes like manganese superoxide dismutase (MnSOD) [31]. Furthermore, this layer is the first line of fetal cells that encounter oxygen-rich maternal blood, thereby being exposed to the highest oxygen levels and increasing their vulnerability to ROS. A rise in ROS induces an imbalance in the oxidant/antioxidant activity, known as oxidative stress. Eventually, in severe cases of oxidative stress, clinical symptoms of PE or early pregnancy failure could result [1].

## 3. Oxidative stress

Although the rise in oxygen tension in the intervillous space is a



**Fig. 1. Schematic representation of the maternal and fetal vasculature in the second trimester placenta.** (A) Macroscopic (simplified) view of the placenta with inset (B) showing detailed morphology of intervillous space, villi and spiral arteries under normal (left of dashed line) and ischemic (right of dashed line) conditions. The normal condition is characterized by well-developed villi (C) covered with villous cytotrophoblast (dark pink, oval) and syncytiotrophoblast (light pink, cubic), containing fetal vessels covered with villous endothelium (yellow). Some red blood cells are present within the vascular lumen. Various Hofbauer cells (green) are depicted. Extravillous trophoblast (EVT) invasion (pink, continuous lining) causes spiral artery remodelling thereby creating low resistant perfusion of the intervillous space with maternal blood. This results in proper oxygenation as indicated by the red colour of the intervillous space. Under ischemic conditions the villi become hypoplastic (D). Villous trophoblast becomes discontinuous; the villous surface is characterized by increased fibrin deposition and increased syncytial knotting. Due to impaired EVT invasion in the first trimester (pink, interrupted lining) spiral arteries are inappropriately remodelled. This results in hampered perfusion of the intervillous space causing ischemia in the intervillous space (indicated by the blue colour of the intervillous space). Ischemia exposes villous tissue to increased oxidative stress and is held responsible for the described morphological changes as depicted in right panel B and panel D.



**Fig. 2. Histology of normal villi in the late second trimester placenta.** Villi are characterized by the formation of vasculosyncytial membranes (VSM) due to clustering of syncytiotrophoblast in syncytial knots. Within the intervillous space (\*) maternal red blood cells are present. Fetal vessels are filled with fetal red blood cells. (H&E staining, original magnification  $\times 400$ ).



physiological phenomenon, it results to some degree in placental oxidative stress. To compensate for this increase in oxidative stress, a rise in antioxidant activity is observed as the placenta adapts to this new high oxygen-rich environment. There is a sharp rise in oxidative stress in the trophoblast associated with the onset of maternal blood circulation in the placenta. This coincides with an increase in placental activity of the antioxidants glutathione peroxidase and catalase in normal pregnancy. It might well be that the gradual opening of increasing numbers of maternal vessels allows the placental tissue to adapt to the increasing oxygen tensions [1].

Formation of ROS is the result of the reduction of molecular oxygen, and ROS are generated for instance by oxidative phosphorylation at the mitochondrial membrane. In normal physiology, ROS is involved in cellular signaling pathways contributing to normal development and cell function [2]. It contributes for example to trophoblast invasion and to vascular development in the placenta. To achieve and maintain physiological ROS levels, there is a dynamic balance between the generation of ROS and the activity of antioxidants to reduce ROS [2].

An example of ROS production is superoxide anion formation ( $O_2^-$ ). Superoxide is produced via enzymatic and non-enzymatic processes that take place in the cytoplasm, peroxisomes and endoplasmic reticulum, but mostly in the mitochondria [18]. In a non-enzymatic manner, leakage of electrons from enzymes of the mitochondrial respiratory chain takes place and reduce molecular oxygen thereby forming  $O_2^-$ . In addition to superoxide, other oxidants are formed such as nitric oxide (NO), hydrogen peroxide ( $H_2O_2$ ) and peroxyxynitrite anion ( $ONOO^-$ ) [20,23].

As a defense mechanism, the enzyme manganese superoxide dismutase (MnSOD) catalyzes the dismutation of  $O_2^-$  and the formation of hydrogen peroxide, which is subsequently converted to oxygen and water by glutathione peroxidase and catalase [1]. Besides MnSOD catalase, other enzymes such as glutathione peroxidase and copper/zinc superoxide dismutase are available in the placenta that function as antioxidants [1]. In the placenta the cytotrophoblasts and the villous stromal cells are able to synthesize new antioxidants when exposed to ROS [32].

However, if the capacity to synthesize new antioxidants is not sufficient to scavenge the excess amount of ROS, oxidative stress results in DNA and protein damage and lipid peroxidation [18]. Protein damage results from oxidation leading to abnormal folding in the endoplasmic reticulum or loss of enzyme/receptor function [18]. DNA is vulnerable to oxidative damage by strand breakages and cross-linkages interfering with chromatin folding, transcription and DNA repair. Lipid peroxidation effects cell function by loss of cell membrane fluidity. These effects could all lead to cell damage and cell death [18].

### 3.1. Oxidative stress in the first trimester placenta

Watson et al. investigated the viability of human syncytiotrophoblast derived from chorionic villous tissue and analyzed the mitochondrial activity and ultrastructure of the villi [32]. They found deterioration of syncytiotrophoblast in the presence of high oxygen levels shown by vacuolization of the cell, decrease of microvilli at the surface and by decrease of mitochondria, without damage to cytotrophoblasts and stromal cells. In another study, the same authors demonstrated that syncytiotrophoblast in early pregnancy expresses low amounts of antioxidants [32,33]. Cytotrophoblasts and villous stromal cells showed higher levels of antioxidants as detected by immunohistochemistry.

Syncytiotrophoblast can adapt to minimal increases in ROS by restoring the oxidant/antioxidant activity balance, which is seen in normal pregnancy. If adaptation in response to oxidative stress is

inadequate, insufficient increase in antioxidative capacity leads to maladaptation of the mitochondrial ultrastructure and intermediate damage of the syncytiotrophoblast. This could result in a state of chronic oxidative stress. At last, damage to syncytiotrophoblast could be that severe that it leads to early pregnancy failure [1].

### 3.2. Oxidative stress in second and third trimester placenta

Oxidative stress is an important factor in many complications during the second and third trimester of pregnancy. As stated above, inadequate EVT invasion could result in an imbalance of oxidant/antioxidant activity when antioxidant capacity does not keep pace with increased oxygen tension leading to a chronic state of oxidative stress.

Another previously described hypothesis explains the oxidative stress by intermittent maternal blood flow in the intervillous space resulting in ischemic-reperfusion damage. Ischemia-reperfusion injury (IRI) is also mediated through generation of ROS by various pathways, for example through mitochondrial electron transfer processes and activity of a variety of enzymes like NADPH oxidase. Generation of ROS is seen in villous endothelium and to a lesser extent in villous stromal cells and syncytiotrophoblast after re-oxygenation *in vitro*. An extensive production of ROS, for example in repeated IRI, leads to irreversible cellular dysfunction and tissue damage [23].

### 3.3. Clinical manifestations of oxidative stress

Oxidative stress can result in several pregnancy complications. PE, the most investigated complication of pregnancy, develops in the second or third trimester of pregnancy, and is characterized by maternal endothelial cell dysfunction resulting in systemic endovascular inflammation. This could cause symptoms like proteinuria and hypertension [3]. Inadequate invasion of EVT in the decidua contributes to the syndrome by causing oxidative stress. Early PE (below 32 weeks of gestation) is often accompanied by fetal growth restriction [3,34], which is the second most studied pregnancy complication.

### 3.4. Adaptation mechanisms of the placenta to hypoxia

It is thought that the placenta can respond to hypoxia by increasing the vascularity of the villi, called villous chorangiogenesis. This diagnosis, which is amongst others associated with FGR and fetal hypoxia, is made if histologic examination shows 10 or more capillaries in 10 or more terminal villi in 10 or more areas of the placenta using a 10× objective [35]. It is suggested that local growth factor production (in response to e.g. diabetes mellitus, smoking, anaemia and living at high altitude) like vascular endothelial growth factor (VEGF) and cytokines enhance this hypercapillarization [36,37]. Stanek found that various types of hypoxia may result in different types of adaptation in placental vascularity. When also lesions indicative of placenta hypoxia like maternal vascular malperfusion (MVM) are encountered, fetal outcome was worse when compared to cases with chorangiogenesis only. It is assumed that chorangiogenesis takes weeks to develop [38], and it is stated that this is a response to chronic hypoxia and therefore diffuse chorangiogenesis is considered an adaptation mechanism to IRI [11].

The fetus can compensate for hypoxia by increasing the amount of circulating nucleated red blood cells (NRBCs) by acceleration of the erythropoietic process. NRBCs in umbilical cord blood in mothers with PE are significantly elevated [39]. In a recent study, a statistically significant increase in NRBCs was found in the rat fetus after 24 h of exposure to hypoxia. It is however unclear whether

this response is similar in the human fetus [40]. Increase in NRBCs is associated with adverse neonatal outcome and hypoxic-ischemic encephalopathy [41].

#### 4. Placenta pathology

##### 4.1. Placenta histology associated with impaired remodelling of spiral arteries

Placental pathology can originate from abnormalities in one of the three vascular compartments of pregnancy, *i.e.* from the maternal circulation, the fetal circulation and from the placental parenchyma itself (Fig. 3, Fig. 4). As stated before, hypoxia of the placenta can result from inadequate remodelling of the spiral arteries, giving rise to decidual arteriopathy. Decidual arteriopathy is histologically characterized by acute atherosclerosis, fibrinoid necrosis with or without foam cells, and mural and intramural endovascular trophoblast persistence in the third trimester [17,42] (Fig. 4B). These changes are generally difficult to find in placental tissue when examined histologically as they are primarily located in the deeper part of the decidua and myometrium, which is normally not attached to the placenta. Spiral arteries can be studied however in placental bed biopsies. Other complicating factors are that spiral artery remodelling is not uniformly distributed across the placental surface, and that spiral artery remodelling is macroscopically not visible [42].

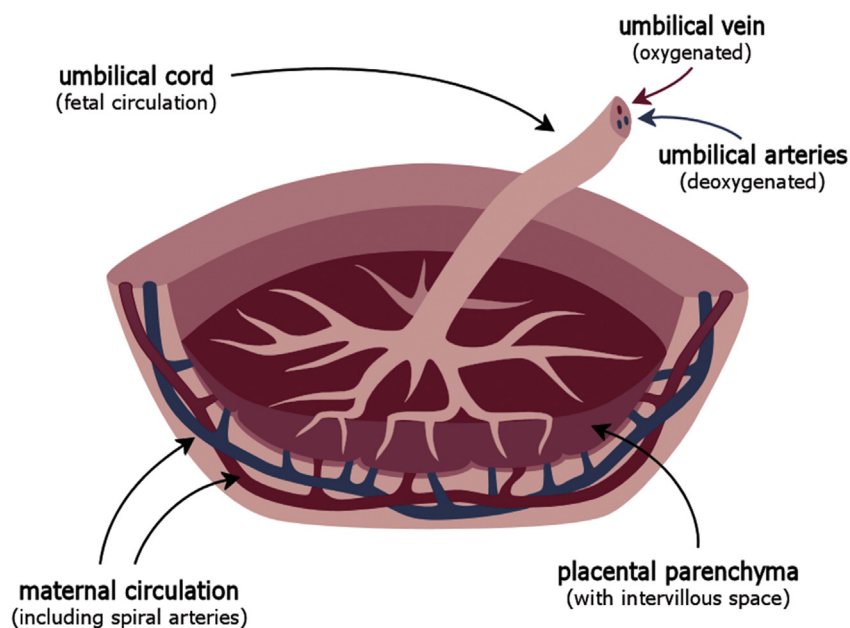
A secondary effect of the inadequate spiral artery remodelling is injury from high velocity blood flow in the intervillous space (Fig. 1B, right half, “ischemic”). This high-velocity malperfusion results in multiple high shear stress lesions associated with MVM and can be recognized both macroscopically and microscopically. Macroscopic findings of MVM include placental hypoplasia (trimmed weight <10th percentile), a narrow umbilical cord (less than 0.8 cm diameter at term), infarction and retroplacental haemorrhage [17,42]. Most commonly, infarction occurs after occlusion of a spiral artery. This leads to cessation of oxygenated blood supply resulting in collapse of the intervillous space. Due to the resulting hypoxia, the trophoblast layer of the villi first loses its viability,

followed by cell death of the villous stromal and vascular cells [42,43]. Microscopic lesions include distal villous hypoplasia and accelerated villous maturation. Distal villous hypoplasia shows large intervillous spaces between thin and elongated villi in the lower two-thirds of a full thickness parenchymal slide, involving at least 30% of the slide [17]. Accelerated villous maturation is characterized by the presence of gestational age hypermature villi, usually coinciding with increased syncytial knotting (Fig. 4C) and increase in intervillous fibrin deposition (Fig. 4D) [17,42,43].

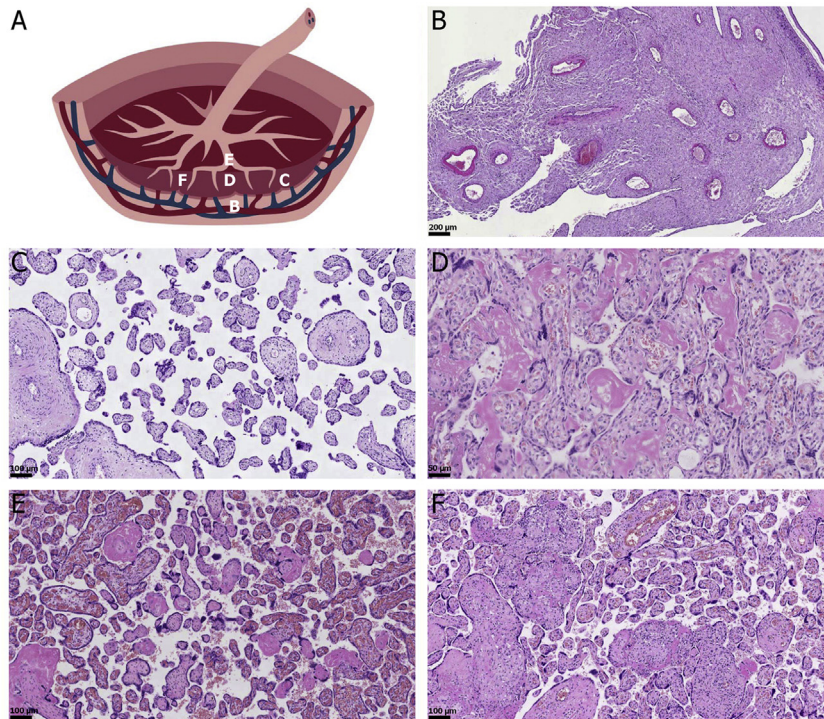
Little information is available on the association between development of placental lesions and the presence of oxidative stress. However, Perrone et al. found a significant increase in F2-isoprostanes (a product of peroxidation) in cord blood in preterm babies (before 32 weeks gestational age) with placental lesions like maternal and fetal vascular malperfusion, compared to preterm babies without placental lesions [44]. Although a lot of research focussed on placental histology associated with impaired remodelling of spiral arteries, some primary placental lesions not related to impaired spiral artery remodelling could cause hypoxia of the fetus. These are discussed in the next paragraph.

##### 4.2. Placental histology associated with fetal hypoxia

Hypoxia of the fetus can also result from primary lesions of the villi itself that decrease diffusion capacity of the VSM, rather than being caused by an impaired maternal blood flow [43]. Such lesions are chronic villitis and avascular villi due to fetal vascular malperfusion reducing the amount of functional villous parenchyma. Fetal vascular malperfusion (FVM) is the result of an obstruction in the fetal blood flow resulting in thrombosis, villous stromal-vascular karyorrhexis and avascular villi (Fig. 4E). This could be caused by a number of lesions like umbilical cord pathology (e.g. hypercoiling of the umbilical cord), hypercoagulability and secondary to acute chorioamnionitis with chorionic vasculitis and/or umbilical funitis or to chronic villitis [17] by inflammation-mediated endothelial damage [45]. Chronic villitis can be the result of viral infections (like the TORCHes), but in most cases no etiology is identified. It is histologically characterized by a lymphohistiocytic infiltrate in the



**Fig. 3. Schematic macroscopic view of the placenta.** The different compartments in which pathology may occur are indicated: the fetal circulation, the maternal circulation, and the placental parenchyma.



**Fig. 4. Examples of placental pathology.** (A) Schematic macroscopic view of the placenta. Characters B to F indicate the locations from which the histological specimens shown in panels B to F were taken. (B) Decidual arteriopathy (H&E staining, original magnification  $\times 50$ ), (C) Distal villous hypoplasia and increased syncytial knotting in maternal vascular malperfusion (H&E staining, original magnification  $\times 100$ ), (D) Increased intra- and perivillous fibrin deposition (H&E staining, original magnification  $\times 200$ ), (E) avascular villi in fetal vascular malperfusion (H&E staining, original magnification  $\times 100$ ), (F) intravillous lymphohistiocytic infiltrate in chronic villitis (H&E staining, original magnification  $\times 100$ ).

villi, sometimes accompanied by plasma cells (Fig. 4F). The infiltrate can damage villous vessels and can cause obliteration of those vessels. The appearance of non-perfused villi culminates in avascular villi eventually, thereby impairing the fetoplacental circulation [17].

## 5. Oxidative stress as biomarker and therapeutic target

### 5.1. Clinical detection of oxidative stress in pregnancy

In current regular clinical practice, the only way to discover oxidative stress is use of the ultrasound and cardiocography to detect the long-term consequences like FGR, PE and fetal distress.

In order to study the role of oxidative stress in placental dysfunction and pathology, sensitive methods for detection of oxidative stress are indispensable. In literature, various methods to detect placental oxidative stress are described. One of the first described oxidative stress biomarkers was malondialdehyde (MDA), a breakdown product of fatty acid oxidation and therefore an indicator of lipid peroxidation. MDA is elevated in the blood of pregnant women who suffer from PE [46,47]. MDA binds to TBARS (thiobarbituric acid-reactive substances), which are also elevated in blood of women with PE [48] or with pregnancy-induced hypertension [7] thus reflecting a status of oxidative stress.

Another way of measuring oxidative stress is by determining levels of fetal cellular mRNA expression of anti-angiogenic and pro-angiogenic factors in maternal blood. It is known that during pregnancy some trophoblast cells and other placenta-derived cellular debris circulate in the maternal blood. These fetal-derived cells are therefore easily available for research [49] and may indicate the pathophysiological state of the placenta.

The cellular levels of fetal/placental mRNA of the anti-angiogenic factors vascular endothelial growth factor receptor-1

(Flt-1), endoglin (ENG), P-selectin and placenta specific-1 (PLAC1), and the pro-angiogenic factors placental growth factor (PlGF) and heme oxygenase-1 (HO-1) have been measured in maternal blood. There is at early second trimester -around 15–20 weeks gestation- a significant correlation between high mRNA expression levels of the anti-angiogenic factors Flt-1, ENG, P-selectin and PLAC1 and the development of PE later in pregnancy. Also, mRNA levels of PlGF and HO-1 were significantly lower in women developing PE compared to women without PE [50]. Although angiogenic biomarkers may be promising for predicting development of PE, a WHO multicenter study published in 2015 found that soluble Flt-1, soluble ENG and PlGF measurements during the first half of pregnancy do not perform well enough to predict development of PE later in pregnancy [51]. This is possible due to the fact that the pathophysiology of PE is too heterogenous and angiogenic factors are only altered in a subset of preeclamptic patients. Further investigation to subtype PE characterized by angiogenic imbalance from other etiologies is therefore warranted [51].

Besides angiogenic biomarkers, gasotransmitters could be used as biomarkers for pregnancy complications related placental insufficiency. Three different gasotransmitters can be identified: nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide ( $H_2S$ ). These are small, gaseous signaling molecules that are able to diffuse through cell membranes and regulate vascular tone.  $H_2S$  not only regulates vascular tone but is also involved in immunological and angiogenic processes and ROS scavenging. In PE and FGR, the bioavailability of NO and CO is impaired [52]. NO is produced through conversion of L-arginine by nitric oxide synthases (NOS). There are different types of NOS. In the placenta endothelial NOS (eNOS) and inducible NOS (iNOS) are expressed by fetal endothelium, syncytiotrophoblasts and extravillous trophoblasts. iNOS is also expressed by Hofbauer cells in the villous stroma. It is



suggested that NO is involved in spiral artery remodelling, and impaired NO production is found during PE [52]. CO is formed during the conversion of heme to biliverdin by heme oxygenase-1 and -2 (HO-1 and HO-2). Like eNOS, HO-1 and HO-2 are expressed by fetal endothelium, syncytiotrophoblasts and extravillous trophoblasts. CO decreases villous vascular tone in isolated human placenta [53]. It is possible that the HO/CO-system influences the angiogenic changes seen in PE [52]. Hydrogen sulfide (H<sub>2</sub>S) can be measured in maternal blood and is known to be decreased in women with PE and FGR. However, the gaseous measurements of H<sub>2</sub>S are widely disputed. H<sub>2</sub>S is the end-product of conversion of L-cysteine by cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE). Like iNOS, CBS is expressed by fetal endothelium, syncytiotrophoblasts and Hofbauer cells. CSE is mainly expressed by fetal endothelium and vascular smooth muscle cells [52]. The different cellular locations of gasotransmitter expression in placental villi is shown in Fig. 5. The contribution of H<sub>2</sub>S to PE however is not yet clarified [54].

## 5.2. Therapeutic options for hypoxic condition

In the last decades, many studies have been performed aiming at the prevention of the burst of oxidative stress during complicated pregnancies. However, most of these studies have focused on the identification of biomarkers for PE in pregnant women without resulting in preventive strategies. Angiogenic biomarkers were found to have limited predictive value for the risk to develop PE. Therefore, other markers like gasotransmitters and ROS are important candidates for further study.

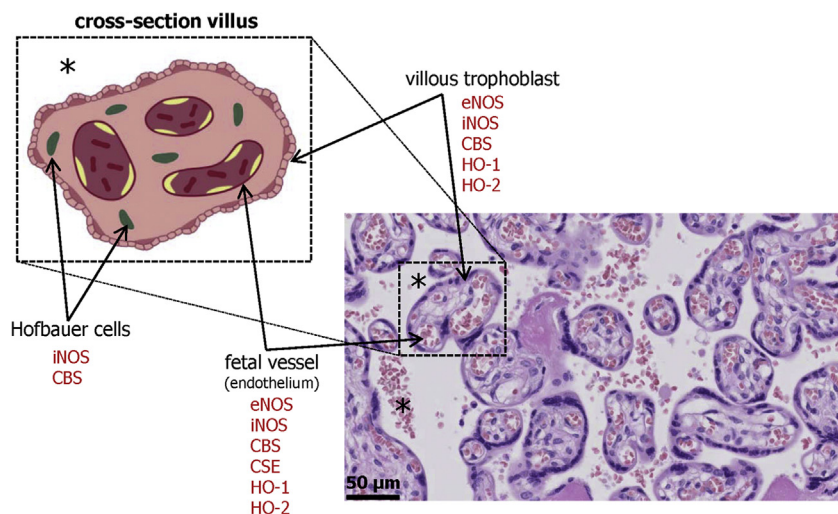
When considering oxidative stress as an important driver of development of placenta pathology, antioxidant therapy might be a suitable candidate to prevent placenta pathology, thereby protecting the fetus and the mother. In literature, most data with antioxidant therapy are obtained in patients with PE and are based on trials with vitamins C and E, however with contradictory results. This might well be related to the fact that ROS also has physiological functions which should not be compromised by treatment [55–60]. In an experimental study in sheep administration of melatonin to pregnant ewes decreased oxidative stress through directly scavenging of ROS and indirectly by increasing the expression of antioxidant enzymes like glutathione peroxidase and superoxide

dismutase (SOD) [61]. Results from various studies give some guidance on how to treat placental hypoxia. For example, in obstructive sleep apnea syndrome (OSAS) oxidative stress is reflected by an increase in thioredoxin and MDA, and a decrease in SOD and reduced iron. Treatment with vitamin C [62] and N-acetylcysteine (NAC) [63] showed a reduction of oxidative stress in OSAS. It is thought that NAC reacts with redox-reactive components of the signaling cascade or acts via scavenging of O<sub>2</sub>-derived radicals. Furthermore, NAC reduces the production of O<sub>2</sub><sup>-</sup> [64]. Wiest et al. showed that NAC transplacental transfer is fast and that fetal NAC exposure occurs rapidly after maternal dosage in cases of chorioamnionitis [65]. Therefore, NAC could be explored as a therapeutic agent in placental oxidative stress.

An increase in bioavailability of gasotransmitters could be another option to reduce oxidative stress. Besides their function as antioxidants, they also have vasodilatory properties [52]. In clinical trials, the use of L-arginine (a NO precursor) resulted in a significant risk reduction of developing PE [58]. The effects of the NO pathway on FGR and PE needs to be studied thoroughly and is currently evaluated in the STRIDER study, an international cohort in which women are randomized for Sildenafil versus placebo in severe and early FGR [66]. Like NO, H<sub>2</sub>S donor therapy could also have beneficial effects on prevention of PE and needs to be studied further.

Pravastatin, a statin used in treatment or prevention of cardiovascular diseases is tested as a potential treatment for PE. Besides blood pressure lowering effects, it also prevented the incidence of FGR by improving the vascular profile in various ways [58]. For example, Kumasawa et al. found a decrease of soluble Flt-1 and an increase in PlGF in mice with PE after administration of pravastatin [67]. A second function of pravastatin is increasing the release of NO and CO, thereby increasing the bioavailability of these gasotransmitters with vasodilatory effects [58]. Lefkou et al. demonstrated beneficial effects of adding pravastatin to the standard treatment of antiphospholipid syndrome (low-dose aspirin and low-molecular weight heparin) on pregnancy outcomes in early onset PE and FGR [68].

Another vasodilator drug is magnesium sulfate (MgSO<sub>4</sub>), used in prevention and treatment of seizures in respectively PE and eclampsia. In the maternal circulation MgSO<sub>4</sub> has a mild vasodilator effect and improves blood flow [59]. Abad et al. showed that MgSO<sub>4</sub> functions as a protective antioxidant in women with severe PE [69].



**Fig. 5. Schematic overview and histology indicating the different cellular locations within placental villi where the enzymes responsible for gasotransmitter (NO, CO and H<sub>2</sub>S) synthesis are expressed.** Abbreviations: CBS: cystathionine-β-synthase; CSE: cystathionine-γ-lyase; eNOS: endothelial nitric oxide synthase; HO: heme oxygenase; iNOS: inducible nitric oxide synthase. Asterisks (\*) indicate the intervillous space. (H&E staining, original magnification ×400).



When administered to women with a risk of preterm birth, MgSO<sub>4</sub> is also neuroprotective for the fetus. It is not known yet if the same is applicable to term fetuses [70].

To develop novel therapeutic agents for treatment or prevention of oxidative stress during pregnancy, understanding the mechanisms regulating placental drug transport is necessary. Different factors influence drug transfer across the placenta, for example, surface area for exchange, blood flow in umbilical and maternal circulation, and the permeability of the placenta in relation to the pharmacotherapeutic agent given [65]. Active as well as passive transport through diffusion of pharmacotherapeutic agents occur from maternal to fetal blood. Both syncytiotrophoblast and fetal endothelium express active drug transporters to maintain the blood-placental barrier. For example, ATP-binding cassette (ABC) transporters, organic anion transporters (OAT), organic cation transporters, serotonin (5-HT) transporter and noradrenalin transporter are found to be expressed in human placental tissue [71]. Of many of these drug transporters polymorphisms have been described [71]. Also, the localization and the role of many drug transporters is still unknown. The function of drug transporters is mediated by hormones like estradiol and progesterone as well as by glucocorticoids. Therefore, the expression of drug transporters could be influenced by infection or inflammation [71]. Besides transport of beneficial medication to the fetus (for instance glucocorticoids to enhance lung maturation in case of premature delivery), the placenta can also function as a barrier for teratogenous, harmful drugs.

## 6. Conclusion

To understand the mechanisms of oxidative stress in different placental lesions we need to broaden the spectrum of pregnancy complications and not only focus on PE. One way of doing so is to associate placental histology/pathology with biomarkers of oxidative stress. These biomarkers can be found in fetal (umbilical cord) blood, in maternal blood and in the placenta. In the future we aim to predict which clinical and placental changes are to be expected based on the level of different types of biomarkers measured. Besides that, it is important to focus on therapeutic agents and their pharmacokinetic features and the effective or optimal concentration in the fetal and/or maternal circulation. Furthermore, it is crucial to also focus on the placental drug transporters they react with. Suitable therapeutic agents need to be able to cross the placental barrier and ideally should not be harmful in any way to the mother and her fetus. With that knowledge, we could try to resolve or prevent oxidative stress and improve maternal and fetal outcome.

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## Declarations of interests

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## Conflicts of interest

There is no conflict of interest.

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