

# The Role of Oxidative Stress in Female Reproduction and Pregnancy

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## 1. Introduction

In a healthy body, reactive oxygen species (ROS) and antioxidants remain in balance. Oxidative stress occurs when the generation of reactive oxygen species and other radical species exceeds the scavenging capacity by antioxidants of antioxidative agents in organism, due to excessive production of reactive oxidagen species and/or inadequate intake or increased utilization of antioxidants. Most ROS are formed at operation of electrone transport chains in mitochondria, endoplasmatic reticulum, plasmatic and nuclear membranes. Minor ROS amounts are generated by some enzymes through autooxidation of different molecules. Reactive oxygen specieses can also be formed by exogenous exposures such as alcohol, tobacco smoke, and environmental pollutants. Elimination of reactive oxygen species is catalysed by certain enzymes such as superoxide dismutases (SOD), catalases and peroxidases. Antioxidants (including vitamins C and E) and antioxidant cofactors (such as selenium, zinc, and copper) are capable to dispose, scavenge, or suppress ROS formation. „Oxidative stress” rises when due to some reasons the steady-state ROS concentration is increased, leading to oxidative modification of cellular constituents, resulting disturbance of cellular metabolism and regulatory pathways (Lushchak. 2011). Cellular ROS and their control by antioxidants are involved in the physiology of the female reproductive system. Physiological ROS levels play an important regulatory role through various signalling and transduction pathways in folliculogenesis, oocyte maturation, corpus luteum, uterine function, embryogenesis, embryonic implantation and fetoplacental development. Imbalances between antioxidants and ROS production are considered to be responsible for the initiation or development of pathological processes affecting female reproductive processes.

The establishment of pregnancy requires a receptive uterus able to respond to a variety of biochemical and molecular signals produced by the developing conceptus, as well as specific interactions between the uterine endometrium and the extra-embryonic membranes. Therefore, placental development and function are prerequisites for an adequate supply of nutrients and oxygen to the fetus and successful establishment of pregnancy. Oxidative stress has been proposed as the causative agent of female sterility, recurrent pregnancy loss and several pregnancy-related disorders as preeclampsia, intra-uterine growth restriction (IUGR) and gestational diabetes.

## 2. Effect of oxidative stress on female reproductive system

The female reproductive system is a complex multiorgan system which require an optimal biological environment. Aerobic metabolism utilizing oxygen is essential for reproductive homeostasis. Aerobic metabolism is associated with the generation of prooxidant molecules called ROS including hydroxyl radical, superoxide anion, hydrogen peroxide, and nitric oxide. The balance between the prooxidants and antioxidants maintain the cellular homeostasis, whenever there is an imbalance in this equilibrium leading to enhanced steady-state level a state of oxidative stress is initiated. Free radicals are key signal molecules modulating reproductive functions by the influence of the endometrial and fallopian tube function, maturation of oocytes, sperm, implantation of the preembryo and early embryo development (Figure 1.)

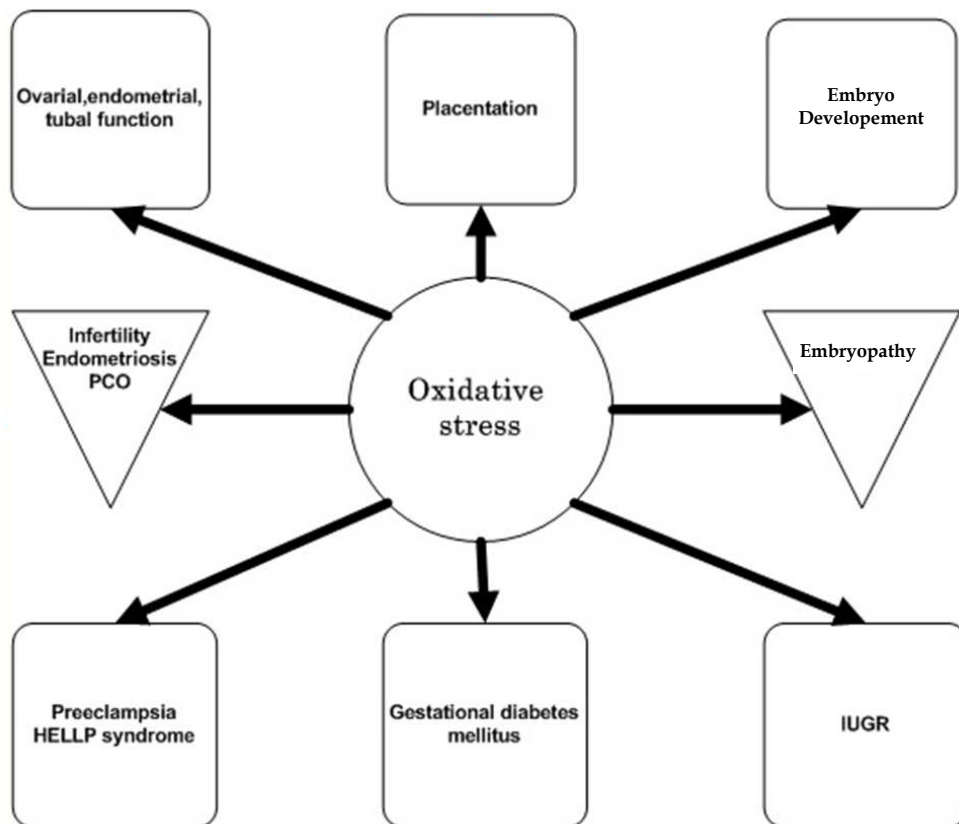


Fig. 1. Oxidative stress in female reproduction

### 2.1 Ovarian function

Aerobic metabolism utilizing oxygen is essential for development of the gametes, also free radicals play a significant role in physiological processes within the ovary. The expression of

various biomarkers of oxidative stress has been investigated in normal cycling human ovaries (Maruyama et al. 1997, Matsui et al., 1996), justifying the regulatory role of ROS and antioxidants in oocyte maturation, folliculogenesis, ovarian steroidogenesis and luteolysis (Shiotani et al., 1991; Behrman et al., 2001; Sugino et al., 2004). Studies demonstrate intensified lipid peroxidation in the preovulatory Graafian follicle (Paszkowski et al., 1995). The significance of reactive oxygen species and antioxidant enzymes as copper zinc superoxide dismutase (Cu, Zn-SOD), manganese superoxide dismutase (Mn-SOD), and glutathione peroxidase, in oocyte maturation was provided by Riley et al. (1991) using immunohistochemical localization and mRNA expression (Tamate et al., 1995). The antioxidant enzymes neutralize reactive oxygen species and protect the oocyte. In corpora lutea collected from pregnant and nonpregnant patients, it was observed that during normal situations Cu-Zn SOD expression rise from early luteal to midluteal phase and decrease during regression of the corpus luteum. Studies investigating the correlation between adrenal-4 binding protein (Ad4BP) and superoxide dismutase expression also suggest an association between oxidative stress and ovarian steroidogenesis (Matsui et al., 1996). Antibody to adrenal 4-binding protein (Ad4BP) was utilized to localize Ad4BP in the nuclei of theca and granulosa cells. Ad4BP is a steroidogenic transcription factor that induces transcription of the steroidogenic P450 enzyme. Both human granulosa and luteal cells respond to hydrogen peroxide with an extirpation of gonadotropin action and inhibition of progesterone secretion (Sabuncu et al., 2001). The production of both progesterone and estradiol hormones is reduced when hydrogen peroxide is added to a culture of human chorionic gonadotropin-stimulated luteal cells (Agrawal et al., 2006).

Levels of three oxidative stress biomarkers, conjugated dienes, lipid hydroperoxide and thiobarbituric acid reactive substances were found significantly lower in the follicular fluid compared with serum levels (Jozwik et al., 1999). The preovulatory follicle has a potent antioxidant defense, which is depleted by the intense peroxidation (Jozwik et al., 1999). Also the antioxidant enzymes glutathione peroxidase and Mn-SOD are considered to be markers for cytoplasmic maturation in metaphase II oocytes (El Mouattasim et al., 1999).

## 2.2 Changes in endometrium

Cyclical changes in the endometrium are accompanied by changes in the expression of antioxidants. Enzymes, such as thioredoxin, have a higher expression in the early secretory phase (Murayama et al, 1997). There is also a cyclical variation in the expression of superoxide dismutase in the endometrium. Superoxide dismutase activity decreased in the late secretory phase while ROS levels increased and ROS triggered the release of prostaglandin F<sub>2</sub> α (Sugino et al., 1996). Estrogen or progesterone withdrawal led to increased expression of cyclooxygenase-2 (COX-2). Stimulation of the cyclooxygenase enzyme is brought about by ROS via activation of the transcription factor NF-κB, suggesting a mechanism for menstruation (Sugino et al., 2004).

Nitrogen monoxide (.NO) has also important role in decidualisation and preparation of the endometrium for implantation by regulation of the endometrial, myometrial and microvascular functions. Expression of endothelial and inducible NO synthase (NOS) have been demonstrated in the human endometrium (Tseng et al., 1996), and the endometrial vessels (Taguchi et al., 2000). Highest levels of transcripts of endothelial NOS mRNA have

been reported in the late secretory phase of the endometrium (Tseng et al., 1996). These changes have been hypothesized to be important in the genesis of menstruation and endometrial shedding.

### **2.3 Fallopian tube function**

Several studies demonstrated the presence of cytokines, prostaglandins, metabolites of lipid peroxidation and ROS in fluid samples of fallopian tube (Tamate et al., 1985). The equilibrium of these components serves as an optimal milieu for fertilization and the transport of the preembryo. An endogenous nitrogen monoxide system exists in the fallopian tubes. Nitric oxide has a relaxing effect on smooth muscle and it has similar effects on tubular contractility. Deficiency of NO may lead to tubal motility dysfunction, resulting in retention of the ovum, delayed sperm transport and infertility. Increased NO levels in the fallopian tubes are cytotoxic to the invading microbes and also may be toxic to spermatozoa (Rosselli et al., 1995), leading to infertility.

### **2.4 Embryo implantation and placenta**

The human embryo undergoes interstitial implantation by invading the maternal decidua at the blastocyst stage (Riley & Behrman, 1991). Although placental villi are bathed in maternal blood in the hemochorial placenta (Tamate et al., 1995), prior to 10 weeks of gestation maternal blood flow to the placenta is blocked by extravillous trophoblasts. Placentation is initiated when the blastocyst makes contact with the epithelial lining of the uterus shortly after implantation. Placental villi which consist of a mesenchymal core surrounded by a monolayer of mononuclear villous cytotrophoblast stem cells which either fuse to form the overlying multinucleated syncytiotrophoblast or, in anchoring villi, differentiate into extravillous trophoblasts which grow out from the villous and spread laterally around the placenta (Irving et al., 1995).

Invasive extravillous trophoblasts play an important role in adapting the decidua to sustain pregnancy. Extravillous trophoblasts invade the walls of the uterine spiral arteries and adapt these vessels into large bore conduits capable of delivering the increased blood supply required in the second and third trimesters (Robertson et al., 1967; Zhou et al., 1997). As the extravillous trophoblasts invade the spiral arteries early in pregnancy they form plugs which occlude the spiral arteries and prevent maternal blood from entering the intervillous space, creating a physiologic hypoxic environment (Hustin & Schaaps, 1987; Jaffe et al., 1997; Burton et al., 1999).

Early placental and embryonic development occurs in a state of low oxygen in histiotroph manner (Evans et al., 2004). The early gestation placenta is poorly protected against oxidative damage, as the antioxidant enzymes Cu,Zn-SOD and Mn-SOD are not expressed by the syncytiotrophoblast until approximately 8-9 weeks of gestation (Watson et al., 1997). Premature perfusion of this space during this first 10 weeks of development increases the risk of pregnancy loss (Jauniaux et al., 2000). The low oxygen environment during early placental development is essential for normal placental angiogenesis, and this angiogenesis is promoted by hypoxia-induced transcriptional and post-transcriptional regulation of angiogenic factors, as vascular endothelial growth factor and placental growth factor (Charnock-Jones & Burton, 2000).

The partial oxygen tension in the intervillous space declines from the second to the third trimester, reaching about 40 mm Hg in the third trimester (Soothill et al., 1986). The exact mechanism by which trophoblasts sense oxygen tension is currently unclear; however, several potential pathways have been identified. Many of these pathways utilize the ROS formation, but it is currently unclear whether hypoxia results in an increase or decrease in their cellular levels (DeMarco & Caniggia, 2002). In hypoxic conditions, trophoblast oxygen sensing mechanisms utilize several different pathways to control gene expression. These pathways often utilize redox-sensitive transcription factors, of which the hypoxia inducible factor (HIF) family are the best characterized. HIF-1 $\alpha$  is a transcription factor and master regulator of the cellular response to low oxygen levels (Majmundar et al., 2010), showing prominent expression in first trimester villi (Wang & Semenza, 1993). HIF-1 $\alpha$  regulates the expression of genes such as p53, p21, and Bcl-2 required for cells to adapt to a low oxygen environment and apoptosis. HIF-1 $\alpha$  is able to be stabilized under normoxic conditions by a variety of growth factors and cytokines including epidermal growth factor (EGF), insulin, heregulin, insulin-like growth factors 1 and 2, transforming growth factor, and interleukin-1 (Zelzer et al., 1998; Feldser et al., 1999; Hellwig-Burgel et al., 1999; Laughner et al., 2001; Fukuda et al., 2002; Stiehl et al., 2002).

Several other transcription factors involved in trophoblast differentiation are responsive to hypoxia. The transcription factors Id1, HIF-1, and HIF-2 (USF1 and USF2) mediate the effects of Mash2, which is up-regulated in 2% oxygen in comparison to 20% oxygen (Jiang et al., 2000; Jiang & Mendelson, 2003). The up-regulation of Mash2, USF1 and USF2 may inhibit cytotrophoblast fusion into syncytiotrophoblast (Jiang et al., 2000; Jiang & Mendelson, 2003). The elevation of intracellular Ca<sup>2+</sup> is believed to activate an HIF-1-independent signalling pathway that involves the transcription factor activator protein-1 (AP-1), with cooperation between the HIF-1 and AP-1 pathways allowing fine regulation of gene expression under hypoxia (Laderoute et al., 2002; Salnikow et al., 2002). AP-1 is a dimeric transcription factor composed from the products of the Jun and Fos proto-oncogenes (c-Jun, JunB, JunD, c-Fos, FosB, Fra-1 and Fra-2) (Dakour et al., 1999). AP-1 transcription factors are believed to play an important role in trophoblast differentiation. In the villus, AP-1 transcription factor expression is limited; however, extravillous trophoblasts express c-Jun, JunB, c-Fos, FosB and Fra-2 both in the first trimester and later in gestation (Bamberger et al., 2004).

The other protective system is formed by antioxidant enzymes, playing a key role in the response of trophoblast to the burst of perfusion by maternal blood. With the increase of oxygen saturation and oxidative stress in the intervillous space the placenta employs a number of physiologic adaptations (Burton, 2009). Levels and activity of antioxidant enzymes: catalase, glutathione peroxidase, manganese and copper, zinc superoxide dismutase are increased within placental tissues. This response is evolved as a defense mechanism to reduce harm to placental tissues exposed to this burst of oxidative stress (Jauniaux et al., 2000).

We can summarize that the trophoblast differentiation is essential for the success of human pregnancy, and despite some conflicting experimental evidence, hypoxia appears to play a vital role in regulating trophoblast differentiation in the first trimester. The regulation of trophoblast differentiation by hypoxia is a result of complex interactions between factors associated with oxidative stress, oxygen sensing mechanisms and the release of

inflammatory cytokines. Therefore, aberrations in any one of these factors, along with the temporal and spatial regulation of blood flow in the intervillous space has the potential to result in altered gene expression and trophoblast phenotype leading to fail of implantation.

### 3. The role of oxidative stress in embryo and fetal malformation

Basic principles of teratogenesis state that a teratogen must cause a specific malformation through a specific mechanism during a period in which the conceptus is susceptible to said mechanism (Karnofsky, 1965). Different mechanisms are responsible for malformations that are in agreement with these basic scientific principles.

A mechanism that has not been well described in teratology is oxidant induced or redox misregulation of developmental signals en route to dysmorphogenesis. The paucity of teratogenic study of redox misregulation is partially due to oxidative stress (Sies, 1985). Oxidizing and reducing equivalent imbalance in turn, leads to macromolecule damage, namely protein modification, lipid peroxidation, and DNA oxidation, and can lead to cell death. Unspecific oxidation of cellular components does not apply to basic principles of teratology or adequately explain the manifestation of teratogenic effects. If oxidative stress is the random, unspecific oxidation of cellular molecules, it does not adequately exemplify why or how specific teratogens that induce oxidative stress could cause a specific malformation. While untimely cell death during differentiation can have serious repercussions on the developing embryo, generalized cellular oxidation and subsequent apoptosis do not sufficiently describe specificity of malformations seen with most teratogens. To qualify as a plausible teratogenic mechanism, oxidative stress must be a controlled, specific event that alters cell function and/ or signal transduction pathways that would in turn cause specific dysmorphogenesis.

During particular periods in development, the embryo is more or less susceptible to oxidative stress. In early development, one-cell embryo relies on the Krebs cycle, whereas the blastocyst relies on glycolysis and anaerobic pathways as does the embryo during early organogenesis. Once the circulatory system is established, there is a higher reliance on oxidative and aerobic metabolism and more ROS are produced by mitochondria. Conversely, more antioxidants are available at this period to counteract and detoxify these reactive oxygen specieses (Hansen, 2006). Over the course of development, the delicate balance between oxidants and antioxidants can be disrupted by exogenous agents that simulate ROS production leading to oxidative stress.

Thalidomide is associated with multiple birth defects, including phocomelia (Lecutier, 1962; Taussig, 1962). The most sensitive organ to thalidomide toxicity is the limb. Although the mechanism of teratogenesis and determinants of risk remain unclear, related teratogenic xenobiotics are bioactivated by embryonic prostaglandin H synthase (PHS) producing reactive oxygen species, which cause oxidative damage to DNA and other cellular macromolecules. Similarly, thalidomide is bioactivated by horseradish peroxidase, and oxidizes DNA and glutathione, indicating free radical-mediated oxidative stress. Furthermore, thalidomide teratogenicity is reduced by the PHS inhibitor acetylsalicylic acid, indicating PHS-catalyzed bioactivation. This appears to be regulated through redox shift resulting from depletion of GSH and increased GSSG in the nucleus, and this may imply

that various transcription factors are affected by thalidomide through redox regulation (Hansen et al., 2001, 2002).

Exposure to the anticonvulsant valproic acid during the first trimester of pregnancy is associated with an increased risk of congenital malformations including heart defects, craniofacial abnormalities, skeletal and limb defects, and most frequently, neural tube defects (NTDs). The mechanisms by which valproic acid induces teratogenic effects are not fully understood, although previous studies support a role for oxidative stress. Valproic acid can alter cell signaling through gene expression changes mediated through histone deacetylase inhibition (Phiel et al., 2001), and is a direct inhibitor of class I and II histone deacetylases. Several laboratories have shown that embryonic histone acetylation levels are increased following exposure to valproic acid (Menegola et al., 2005; Tung and Winn, 2010). Furthermore, studies have supported a role for histone deacetylase inhibition as a mechanism of teratogenesis as analogs of valproic acid that lack histone deacetylase inhibitory activity are less teratogenic (Gurvich et al., 2005). Gene microarray studies have also demonstrated that valproic acid targets genes regulated by histone deacetylase, including *Mt1* and *Mt2*, both of which are ROS-sensitive (Jergil et al., 2009). In addition, histone deacetylase inhibitors have also been shown to increase ROS production and induce apoptosis in several cancer cell lines (Carew et al., 2008). Therefore, alterations in gene expression and/or increases in ROS formation mediated by histone deacetylase inhibition during development may induce teratogenesis.

The widely used anticonvulsant, phenytoin, can double the incidence of structural and functional birth defects when used in pregnancy (Kaneko et al., 1991). It can induce vascular disruption, which leads to hypoxia and hypoperfusion (Danielsson et al., 1995). In addition, phenytoin results in oxidative DNA damage and dysmorphogenesis, which can be eliminated by antioxidants (Winn and Wells, 1995). Phenytoin also selectively increased NF- $\kappa$ B activity in targeted tissues. Blocking of these signaling events with p65 antisense oligonucleotides eliminated the associated embryopathies (Kennedy et al., 2004). Further evidence that oxidative stress is important in phenytoin mediated toxicity is exemplified by the fact that treatment with polyethylene-modified superoxide dismutase enhances embryo toxicity whereas antioxidant levels were modulated with phenytoin (Winn and Wells, 1999).

Chronic ethanol consumption can lead to the generation of ROS and, as a consequence, teratogenicity. Prolonged ethanol exposure lead to increased production of lipid peroxides and decreased expression of antioxidant enzymes. Ascorbic acid can prevent against ethanol toxicity through inhibition of ROS formation and NF- $\kappa$ B activation (Peng et al., 2005). Zebrafish embryos exposed to ethanol with lipoic acid and/or only partially attenuated ethanol embryo toxicity, suggesting that other mechanisms are also involved (Reimers et al., 2006).

## 4. Oxidative stress in pathological pregnancies

### 4.1 Preeclampsia

Preeclampsia is a complex multisystem disorder that occurs during the pregnancy. The disease affects 5-8% of all pregnancies and is one of the leading causes of maternal and fetal

morbidity and mortality. It is characterized with hypertension and proteinuria. The systolic blood pressure  $\geq 140$  mmHg, diastolic blood pressure  $\geq 90$  mmHg and the proteinuria at least 300 mg in 24 h urine collection. Women with mild proteinuria generally have no symptoms. However, women with severe preeclampsia (blood pressure  $\geq 160/110$  mmHg, proteinuria  $>2.5$ g/24h) may have symptoms such as renal insufficiency, liver disease, haematological and neurological disturbances. Preeclampsia is characterized by vasospasm, reduced placental perfusion and abnormal placentation. The main cause of fetal compromise is the disturbance in uteroplacental perfusion. The only cure is the delivery of the baby. With antihypertensive treatment may prolong the pregnancy, increasing the chances of the baby to survive. If the blood pressure cannot be controlled, or the laboratory parameters entry in a critical value the baby must be delivered. Preeclampsia has been proposed as a two-stage disorder. In first stage the placenta produces cytotoxic factors, in the second stage the maternal response to the placental factors occurs. There are several theories regarding to the main cause of disorder: abnormal placentation, immunological background, abnormal inflammatory response, etc. Assuming preeclampsia literature we can conclude that all the theories are part of disorders etiology.

#### 4.1.1 Oxidative agents

Some researches suggest that the placental oxidative stress may be involved in the etiopathogenesis of preeclampsia. As there was mentioned above, the oxidative stress is described as an imbalance in the production of reactive oxygen species and the ability of antioxidant defense to scavenge them. Pregnancy is a state of oxidative stress arising from the increased metabolic activity in the placenta and reduced scavenging power of antioxidants (Wisdom et al. 1991). During the gestation the oxygenation of the uteroplacental unit is changing. The placenta and fetus exist in a hypoxic environment during early pregnancy as the uterine oxygen tension is extremely low till 8-10. weeks of gestation ( $pO_2 < 20$  mmHg, 5%  $O_2$ ), prior to establishment of the blood flow into intervillous space. The onset of blood flow is proceeding from the periphery to the center of placental disc, with villous regression in the placental periphery involving into the chorion leave (Jauniaux et al., 2003). The developing chorioallantoic villous trees are exposed to a marked increase of  $pO_2$  in a range of 40-80 mm Hg (Sjostedt et al., 1960; Shaaps et al., 2005; Rooth et al., 1961). This reoxygenation of the uteroplacental unit results an oxidative burst, controlled by antioxidant mechanisms.

Proposed effect of oxidative stress on placental fatty acid metabolism.

As a consequence of abnormal trophoblast invasion, and maternofetal barrier preeclampsia is characterized by induced oxidative stress and decreased antioxidants (Patil et al., 2009). In preeclamptic women, maternal circulating levels, placental tissue levels and production rate of lipid peroxides are increased and several antioxidants are markedly decreased (Serdar et al., 2003; Orhan et al., 2003). Normal pregnancy is associated with physiological hyperlipidemia (Belo et al., 2004). Physiological alterations are manifested by increased levels of triglycerides and cholesterol in pregnancy, which decreases rapidly after delivery. Preeclampsia is characterized by further elevation of serum triglycerides and serum free fatty acids (Hubel et al., 1996). (Figure 2.) Increased lipid peroxidation has been reported in preeclampsia, IUGR (Liu et al., 2005; Gupta et al., 2004, Bretelle et al., 2004).



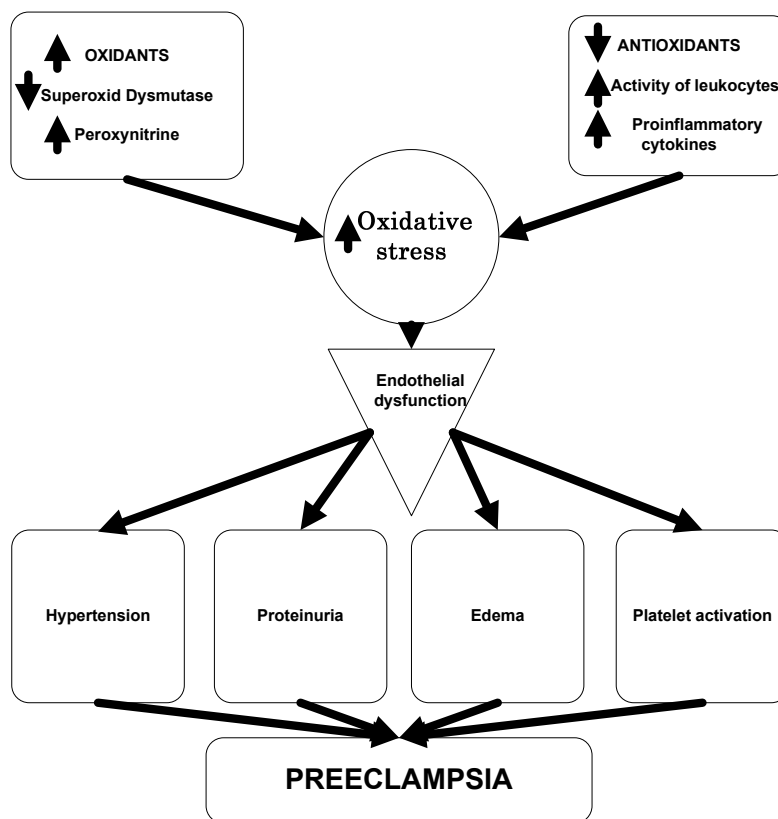


Fig. 2. Supposed oxidative stress pathway in preeclampsia

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is an important enzyme that generates superoxide anion radical localized in the placenta syncytial microvillous membrane (Matsubara & Sato, 2001; Rajmakers et al., 2004). NADPH oxidase may play a role in placental lipid peroxidation by generating increased amounts of the superoxide anion radical. Poor antioxidant reserves can also tilt the balance in favor of prooxidation. Lipid peroxidation results in formation of primary lipid peroxidation products such as lipid hydroperoxides and secondary products such as malondialdehyde (MDA) and lipid peroxides. Lipid hydroperoxides are formed and bind to lipoproteins. They are then carried to distant sites where the hydroperoxides can cause ongoing lipid peroxidation and result in systemic oxidative stress. Increased placental production of lipid peroxides and thromboxane was demonstrated from both the trophoblast and the villous core components of placentas in patients with preeclampsia (Walsh & Wang, 1995).

Studies of women undergoing cesarean section showed significantly higher contents of lipid hydroperoxides, phospholipids, cholesterol and free 8-iso-prostaglandin F<sub>2α</sub> (8-iso-PGF<sub>2α</sub>), but not the total (free plus esterified) 8-iso-PGF<sub>2α</sub> in decidual tissues from women with preeclampsia as compared with tissues from normal pregnancies (Staff et al., 1999).

Moreover, tissue levels of free and total 8-iso-PGF $2\alpha$  are significantly higher in preeclamptic placenta than in normal placenta (Walsh et al., 2000). Isoprostanes like 8-iso-PGF $2\alpha$  are produced specifically by free radical-catalyzed peroxidation of arachidonic acid (Morrow et al., 1990). Free 8-iso-PGF $2\alpha$  has activities of relevance to preeclampsia, being a potent vasoconstrictor in kidney (Morrow et al., 1990) and placenta (Kwek et al., 1990), platelet activator (Minzu et al., 2002), and inducer of the release of endothelin from endothelial cells (Yura et al. 1999).

An increase in diastolic pressure correlates significantly with an increase in lipid peroxide levels, indicating that the severity of hypertension is correlated with the extent of lipid peroxidation (Aydin et al., 2004; Jain & Wise, 1995; Gupta et al., 2006). Women with preeclampsia have significantly higher mean plasma levels of malonaldehyde and significantly lower superoxide dismutase levels compared with normotensive pregnant women, (Aydin et al., 2004). The decrease in nitric oxide (NO) and superoxide dismutase (SOD) levels followed by a concomitant increase in levels of malonaldehyde, fibronectin, endothelin-1 (ET-1), and soluble-E selectin (sE-selectin) correlate with an increase in diastolic blood pressure (Aydin et al., 2004). In further studies, malonaldehyde levels in maternal erythrocytes were significantly elevated in women with developed preeclampsia. The risk of developing preeclampsia was 24-fold higher when malonaldehyde levels were above the cutoff value of 36 nmol/g (Basbug et al., 2003).

The cord plasma malonaldehyde and vitamin E levels were higher in patients with eclampsia than in patients with preeclampsia and in normotensive pregnant patients (Bowen et al. 2001).

Hyperhomocystinemia and altered eicosanoid synthesis has also been implicated in the pathophysiology of preeclampsia. Eicosanoids have vasoactive properties and enhance lipid peroxidation and decrease prostacyclin synthesis. The generation of the eicosanoid, 15-hydroxyeicosateranoic acid by the placenta was higher in women with preeclampsia than in normotensive control subjects. In preeclampsia, there is increased synthesis of thromboxane and reduced synthesis of prostacyclin. Lipid peroxides may also stimulate the cyclooxygenase enzyme to produce more thromboxane, resulting in a hypercoagulable state (Walsh, 2004).

#### 4.1.2 Antioxidant agents

Antioxidants can be enzymatic or nonenzymatic. The enzymatic antioxidants are superoxide dismutase, thioredoxin, thioredoxin reductase, and glutathione peroxidase. The nonenzymatic antioxidants can be lipid-soluble such as vitamin E or water-soluble such as vitamin C. Serum levels of vitamin E and beta carotene (Serdar et al., 2003; Akyol et al., 2000), serum coenzyme Q10 and tocopherol levels (Palan et al., 2004), ascorbic acid were significantly reduced in pregnancies complicated by mild or severe preeclampsia, and the total antioxidant capacity was significantly reduced in pregnant women with mild and severe preeclampsia (Sagol et al., 1999). The balance between lipid peroxides and antioxidant vitamin E is tipped in favor of lipid peroxides in patients with mild and severe preeclampsia. A two-fold increase in the ratio between lipid peroxidation and antioxidant capacity was reported in the antepartum period in women with preeclampsia (Davidge et al., 1992). Significantly lower levels of vitamin C, E, and total thiol were seen in women with

preeclampsia (Kharb et al., 2000). In patients with preeclampsia, antioxidants scavenge the increased free radicals, resulting in lowered antioxidant levels. Water-soluble antioxidants may function as a first line of antioxidants to scavenge excess of reactive oxygen species in plasma, whereas lipid soluble antioxidants such as tocopherol and carotene scavenge reactive oxygen species affecting the membrane lipids (Mikhail et al., 1994).

The activities of placental superoxide dismutase and glucose-6-phosphate dehydrogenase are decreased in preeclampsia compared to normal pregnancy (Poranen et al., 1996). Moreover, the activity and mRNA expression of Cu,Zn-SOD, glutathione peroxidase, and tissue levels of vitamin E are significantly lower in placental tissues from preeclampsia than from normal pregnancy (Wnag & Walsh, 1996). Glutathione and its related enzymes are antioxidants that help detoxify the increased generation of free radicals. Significantly reduced whole blood glutathione levels have been reported in women with preeclampsia and HELPP syndrome (Madazil et al., 2002; Knapien et al., 1998).

#### **4.1.3 Leukocyte activation**

The other main characteristics of preeclampsia is the exacerbated inflammatory state (Bretelle et al., 2004; Holthe et al., 2005; Redman et al., 1999). Activated leukocytes, both monocytes and granulocytes, generate excess reactive oxygen specieses resulting in oxidative stress (Holthe et al., 2004). Compared with normotensive pregnant women, women with preeclampsia have higher levels of calprotectin, a protein involved in various physiological inflammatory processes, which is indicative of leukocyte activation (Holthe et al., 2005). The expression of surface adhesion molecules on cord blood neutrophils was significantly higher in infants born to women with preeclampsia than in infants born to the control subjects. Increased TNF secretion by leukocytes was detected in blood from patients with preeclampsia, providing further evidence of leukocyte activation (Beckman et al., 2004). TNF- $\alpha$  can activate the endothelial cells and upregulate the gene expression of numerous molecules such as platelet-derived growth factor, cell adhesion molecules, endothelin-1 and PAI-1. These molecules have been reported to have detrimental effects on the vasculature and also characterize preeclamptic pregnancy (Hajjar et al., 1987; van Hinsbrgh et al., 1988). Furthermore, chronic infusion of TNF- $\alpha$  into rats during late pregnancy results in a significant increase in renal vascular resistance and arterial pressure (Alexander et al., 2002; Giardina et al., 2002).

#### **4.1.4 Endothelial cell dysfunction**

Endothelial dysfunction is also one of the main pathogenic features of preeclampsia. The markers of endothelial dysfunction such as tissue plasminogen activator, von Willebrand factor, sE-selectin, and fibronectin are elevated in patients with preeclampsia (Aydin et al., 2004; Stubbs et al., 1984; Halligan et al., 1994). Although the exact mechanisms of vascular endothelial damage in preeclampsia are unclear, increased lipid peroxidation may lead to endothelial cell dysfunction (Davidge et al., 1996). Tumor necrosis factor (TNF), tissue factor (TF) of placental origin, endothelial nitric oxide synthase (NOS), and excessive activity of the enzyme polymerase may contribute to endothelial dysfunction. Compared with normotensive pregnant women, women with preeclampsia have reduced expression of constitutive nitrite oxidative stress -mRNA, and this lead to reduced production of NO.

NOS inhibition consequence is the increased endothelial permeability and an abnormal response of the endothelial cells to the stress (Wang et al., 2004). In preeclampsia greater nitrotyrosine immunostaining were found in placental villous vascular endothelium and its surrounding smooth muscle cells, and also in villous stromal cells compared to normal pregnant controls (Myatt et al., 2006). Moreover particularly intense immunoreactivity of nitrotyrosine was measured within the invasive cytotrophoblasts in placental biopsies and vascular endothelium in the floating villi obtained from women with preeclampsia (Many et al., 2000).

#### **4.1.5 Vascular development**

Aberrant placental vasculature development and abnormal placental blood flow are characterized by increased impedance in Doppler velocimetry (Frag et al., 2004). These abnormalities significantly correlated with expression of tissue factor in the placenta of women with severe preeclampsia (Di Paolo et al., 2003). The expression of tissue factor was found to be markedly increased in the endothelial cells within the basal deciduus. Doppler impedance modifications were significantly correlated to the endothelial cell activation. Tumor necrosis factor, a circulating cytokine, has also been implicated as causing endothelial dysfunction in preeclampsia (Hung et al., 2004). Significantly higher tissue levels of tumor necrosis factor were demonstrated in the placenta from women with preeclampsia (Wang et al., 1996). Higher levels of tumor necrosis factor lead to increased generation of E-selectin, a marker of endothelial activation of umbilical endothelial cells.

#### **4.2 Oxidative stress in gestational diabetes (GDM)**

Gestational diabetes is defined as a carbohydrate intolerance of variable severity, which begins, or is identified during the pregnancy (Lopez et al. 2011). The prevalence of gestational diabetes mellitus is around 5% of all pregnancies (Ben Haroush et al., 2004). The presence of this disease increases the risk of macrosomia, perinatal morbido-mortality (Ostlund et al., 2003) and subsequent developement of type 2 diabetes mellitus. The pathophysiology of gestational diabetes remain unclear. Pregnant women with gestational diabetes have a reduction in insulin sensitivity (Catalano et al., 1999), hyperglycemia, and hyperlipidemia. Oxidative stress implication in developement of the disease is a result of imbalance between the increase in the formation of reactive oxidative substances (Brownlee, 2001; Maddux et al., 2001) and the insufficiency of antioxidative defence mechanisms (Chen et al., 2003).

##### **4.2.1 Induction of oxidative stress in gestational diabetes pathways**

Hyperglycemia induces oxidative stress and cell and tissue damage through several metabolic mechanisms.

These include the formation of advanced glycation endproducts (AGE), activation of protein kinase C (PKC), the hexosamine pathway, and increased reactive oxygen species production in the mitochondria. An important source of free radicals in diabetes is the interaction of glucose with proteins. Maillard reaction that form by nonenzymatic glycation through covalent attachment of highly reactive aldehyde or ketone groups of reducing

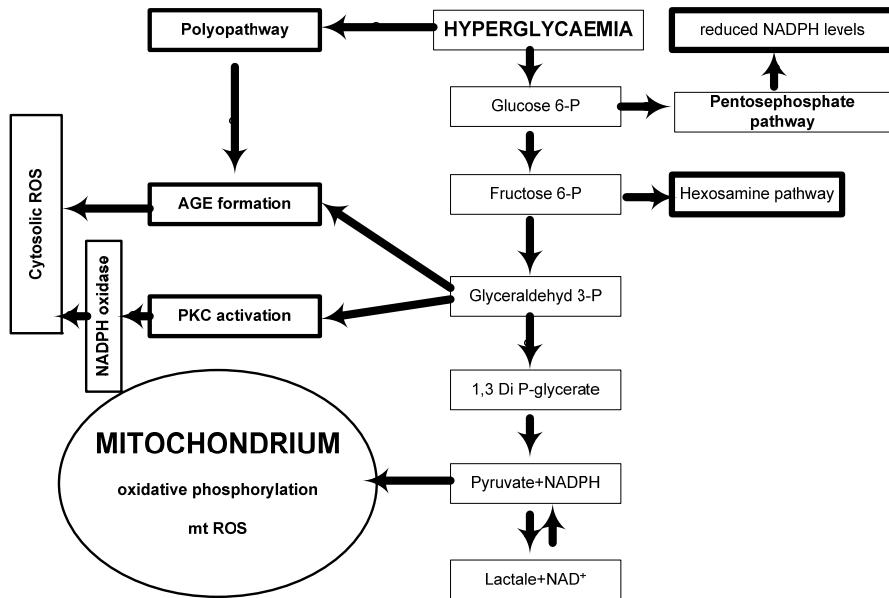


Fig. 3. Mechanisms by which hyperglycemia induces cellular dysfunction and damage.

carbohydrates and the free amino groups on proteins, lipids, and nucleic acids. Elevated concentration of glucose leads to enhanced formation glycolytic products which together with the tricarboxylic acid (TCA) cycle intermediates provide glycation of intracellular proteins. The interaction of aldehyde groups of glucose with free amino groups on proteins generates a Schiff's base. Extracellular AGE can bind to the AGE receptor (RAGE), a multi-ligand member of the immunoglobulin superfamily. Engagement of RAGE by AGE results in activation of intracellular signaling molecules resulting in oxidative stress and inflammation. Since oxidative stress induction and inflammation are closely associated with gestational diabetes (Coughlan et al., 2001; Lappas et al., 2004), it is plausible that the AGE-RAGE system could play a role in the pathogenesis of this metabolic disease. During pregnancy, the AGE-RAGE axis may be involved in oxidative and inflammatory responses. Specifically, AGE-BSA stimulated the release of the pro-inflammatory cytokines IL-1 $\beta$ , IL-6, IL-8, and tumor necrosis factor (TNF)- $\alpha$  and prostaglandins PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub> . NF- $\kappa$ B and MAPK activate expression of several pro-inflammatory genes, including pro-inflammatory cytokines, the adhesion molecules vascular cell adhesion molecule (VCAM)-1, and intercellular cell adhesion molecule (ICAM)-1, and RAGE causing cellular inflammation. This is consistent with gestational diabetes being closely associated with low-grade inflammation (Kirwan et al., 2002) and atherosclerosis (Anastasiou et al., 1998; Hannemann et al., 2002). Additionally, the activation of cytokines by AGE in human placenta may also be involved in insulin resistance associated with gestational diabetes (Colomiere et al., 2009).

Hyperglycemia also activates hexosamine biosynthetic pathway (Rajapakse et al., 2009). This pathway of glucose metabolism uses fructose-6-phosphate derived from glycolysis to metabolize glucosamine-6-phosphate by glucosamine-6-phosphate amidotransferase.

Glucosamine-6-phosphate is a competitive inhibitor of glucose-6-phosphate dehydrogenase (G6PDH). Glucosamine-6 phosphate produced in the hexosamine pathway, leads to decreased NADPH concentrations, diminished cellular GSH levels, and elevated oxidative stress. The activity of G6PDH also rapidly increases in response to intracellular reactive oxygen species production (Jian et al., 2003).

NADPH oxidase is a membrane enzyme complex accounting for ROS generation by electron transport chain and the enzyme is especially important in redox signaling. Under diabetic conditions it can be stimulated by AGE, insulin, and angiotensin II. Hypoxia possibly induces all these stimuli, which can activate NADPH oxidase. Once activated in response to high glucose levels, NADPH oxidase catalyzes the transfer of electrons from NADPH to molecular oxygen to produce  $O_2^-$  converted to  $H_2O_2$ . High glucose levels lead to generation of reactive oxygen species by stimulation of NADPH oxidase (Gao et al., 2009; Gupte et al., 2010).

Under physiological conditions reactive oxygen species are eliminated by cellular defense mechanisms, including diverse enzymes and vitamins. Imbalance of reactive oxygen species production and antioxidant systems of a cell can lead to an upregulation of expression of antioxidant enzyme encoding genes. Hyperglycemia causes excessive reactive oxygen species formation, thus activating the Nrf2/ARE pathway (Xue et al., 2008). NADPH oxidase was shown to be higher expressed and activated in endothelial cells of pregnant women with GDM (Sankaralingam et al., 2009). Protein kinase C (PKC) promotes the activation of mitochondrial NADPH oxidase, thereby leading to oxidative stress events. Once stimulated, NADPH oxidase reduces glutathione levels and impairs the cellular antioxidant defense systems (King et al., 2004).

#### 4.2.2 Oxidant species

Maternal MDA levels in serum and plasma are increased in gestational diabetes mellitus women compared to normal glucose tolerant (NGT) pregnant women (Chaudhari et al., 2003; Surapaneni et al., 2008). Higher levels of lipid peroxidation are evident in patients with poor glycemic control. Proteins undergo oxidative damage, they become increasingly susceptible to proteolytic degradation. Erythrocytes contain proteolytic enzymes that can degrade oxidatively damaged proteins such as hemoglobin, thus preventing the accumulation of nonfunctional proteins and protein fragments. GDM is associated with higher levels of maternal erythrocyte proteolytic activity than NGT controls (Kamath et al., 1998).

#### 4.2.3 Antioxidants

The level of total superoxide dismutase in placental tissues of gestational diabetes mellitus (both diet- and insulin-controlled) patients is lower (Kinalski et al., 2001) or did not significantly change (Biri et al., 2006; Lappas et al., 2010). Relative ratio of Cu,Zn-SOD to 8-isoprostane or protein carbonyl was lower in gestational diabetes mellitus placentas, suggesting that the increase in superoxide dismutase is not sufficient to compensate for the developed oxidative stress (Coughlan et al., 2004).

Oxidative stress plays a significant role in both NO overproduction and loss of NO bioavailability (Gloire et al., 2006; Xia et al., 2007). Oxidative stress leads to NOS-dependent

increases in NO production in different tissues. Reduction in NO-induced stress is related to diabetes-induced endothelial dysfunction, NOS activation can also be induced by diabetes. NO induces the expression of antioxidant enzymes Mn- and Cu,Zn-SODs, and heme oxygenase-1 and increases intracellular glutathione concentration (Moellering et al., 1999). Although NO stimulates  $O_2^-$ -induced lipoperoxidation in membranes, it can also mediate protective reactions to inhibit  $O_2^-$  and  $ONOO^-$  induced lipoperoxidation (Rubbo et al., 1994). NO production has been found increased in the placenta, placental veins and arteries, and in umbilical vein endothelial cells from estational diabetes mellitus patients (Figueroa et al., 2000; vonMadach et al., 2003). NOS expression is also altered, as NOS has been found overexpressed in the placenta and eNoxidative stress increased in umbilical vein endothelial cells from estational diabetes mellitus patients (SanMartin et al., 2006). In estational diabetes mellitus, increases in reactive oxygen species and NO production, evident in the placenta and umbilical vessels, lead to peroxynitrite formation. In platelets from estational diabetes mellitus patients, elevated NOS activity and peroxynitrite production have been reported, possibly associated with platelet dysfunction and membrane damage due to increased lipid peroxidation (Mazzanti et al., 2004). Strong protein nitration is found in term placentas from diabetic rats. Collectively, these data provide evidence of reactive nitrogen species (RNS)-induced damage in estational diabetes mellitus in the placenta and the vasculature of the mother, the placenta, and the umbilical cord, produced as a resulting consequence of exacerbated NO and ROS production.

#### 4.2.4 Role of oxidative stress in gestational diabetes-induced teratogenesis

Diabetes in pregnancy is associated with suboptimal decidualization (Garris et al., 1988). NO plays a key role in decidualization and embryo implantation (Norwitz et al., 2001). It increases vascular permeability, vasodilation, and blood flow in the uterus, and is a component of the decidual cell reaction (Valdes et al., 2009). Diabetes during pregnancy is associated with embryonic dysmorphogenesis. Due to its capacity to regulate cell survival, apoptosis, differentiation, oxidative and nitrosative stresses play a significant role in embryo organogenesis. Low and high levels of NO can lead to embryonic maldevelopment, possibly due to an improper regulation of apoptotic events. During embryo and fetal development, NO has been found to be relevant in regulating differentiation of lung branching morphogenesis, cephalic morphogenesis, heart development, and nephrogenesis (Bloch et al., 1999; Tain et al., 2010).

Transcription factors, as paired box (PAX)-3 and peroxisome proliferator-activated receptor (PPAR)  $\delta$ , has been found to be involved in the induction of both neural tube and heart malformations, the most common malformations in gestational diabetes pregnant (Higa et al., 2007; Loeken et al., 2006). Different antioxidants such as  $\alpha$ -tocopherol and glutathione ethyl ester increase expression of PAX-3 and prevent apoptosis and the induction of hyperglycemia-induced neural tube and heart defects (Chang et al., 2003; Morgan et al., 2008). The higher 8-isoprostane levels observed in the offspring of diabetic animals (Wentzel & Eriksson, 2002; Wentzel et al., 1999) have its own teratogenic potency. Diabetic embryopathy is also associated with inhibition of GAPDH activity resulting from an excess of reactive oxygen species in the embryo (Wentzel et al., 2002). Oxidative glucose metabolism is low and about 80% of the glucose used by the placenta. The effect of oxidative stress on placental glucose metabolism is not known. However, in nongestational tissues,

there is certainly evidence demonstrating oxidative stress regulates GLUT-1 and/or GLUT-3 dependent glucose uptake and transport. On the other hand estational diabetes mellitus placenta is less sensitive to oxidative stress due to the heightened level of antioxidants. Under normal conditions, physiological levels of reactive oxygen species promote and stimulate adequate insulin signaling. The insulin signaling pathway leads to low levels of reactive oxygen species production itself and ROS act as second messengers of which disposal impairs insulin signaling. Insulin-induced reactive oxygen species production is accounted for by activation of the NADPH oxidase NOX4 through PI3K. The reactive oxygen species pathway subsequently activates kinases or induces gene expression by redox-sensitive transcription (Omroy, 2007).

### 4.3 Intrauterine Growth Restriction (IUGR)

Fetal growth depends on the interactions of genetic and epigenetic determinants functioning against an environment of maternal, fetal, and placental influences (Gardosi *et al.*, 1992). Intrauterine growth restriction (IUGR) manifests as a variable syndrome of suboptimal growth and body disproportions rather than a well-defined etiologic entity. Causes for IUGR are diverse and include aneuploidies, non-aneuploid syndromes, infections, metabolic factors and placental disorders. IUGR places the fetus and neonate at risk of death or disability in the perinatal period (Baschat *et al.* 2000; Bernstein *et al.* 2000) and predisposes the child to a lifelong increased risk for hypertension, cardiovascular disorders and renal disease, among others (Murphy *et al.*, 2006). A common definition is an estimated fetal weight less than the 10th percent for gestational age. Diminished fetal arterial and venous Doppler flows in key vascular beds predict worsening fetal acid base status (Rizzo *et al.*, 2001; Baschat *et al.* 2004) and such findings frequently lead to delivery of a markedly premature baby to avoid *in utero* demise.

A diverse number of stimuli and mediators contribute to the observed injury to the chorioallantoic villi but oxidative stress is high on the list as an injurious agent (Hung *et al.*, 2002). The production of reactive oxygen species during oxidative stress is linked with tissue injury in many diseases (Ryter *et al.*, 2007). Paper shows that the placentas of pregnancies with IUGR exhibit overt signs of oxidative stress, with reduced protein translation and particular reductions in key signalling proteins pathways (Yung *et al.*, 2008). Moreover, the syncytiotrophoblast shows signs of endoplasmic reticulum (ER) stress by activating the unfolded protein response, which leads to an ER signal for enhanced apoptosis. The identified dysregulation of protein translation, signalling pathways and trophoblast turnover in placentas of pregnancies with IUGR (Burton *et al.*, 2009). Hypoxia, ischaemia/reperfusion, or both may contribute to placental injury through mechanisms other than reactive oxygen species generation, as variable blood flow to organs also activates the complement cascade (Levy *et al.*, 2000; Hung *et al.*, 2002; Heazell *et al.*, 2008). Activation of the complement cascade injure the fetoplacental unit (Girardi *et al.*, 2003).

Clarifying the role of complement activation in pregnancies complicated by IUGR, and in placental dysfunction generally, may lead to new approaches to treatment for IUGR, as therapeutic options to modulate complement receptors and complement activity are on the horizon.



## 5. Role of antioxidant supplementation in pregnancy

Clinical and research centers are investigating the usefulness of antioxidant supplementation and their role in prevention of pathological pregnancies. Antioxidant supplementation, for example vitamin C and vitamin E, has been shown to have beneficial effects in preventing luteal phase deficiency and resultant increased pregnancy rate (Hemi et al., 2003; Crha et al., 2003). Meta-analysis investigating the intervention of vitamin-C supplementation in pregnancy was inconclusive (Rumbold et al, 2005). Another meta-analysis of women taking any of the vitamin supplements started prior to 20 weeks' gestation revealed no reduction in total fetal losses, or in early and late miscarriage, having used the fixed-effects model. Improved pregnancy rates were also reported with combination therapy with the antioxidants pentoxifylline and vitamin-E supplementation for 6 months in patients with thin endometria who were undergoing in vitro fertilization with oocyte donation (Ledee-Bataille et al., 2002). Supplementation with vitamin E has also been reported to prevent the deleterious effects of ethanol toxicity on cerebral development in the animal model (Peng et al., 2005). There are essential differences among the population groups and the dosage and duration of supplementation for prevention of preeclampsia. Although many advances are being made in the field of antioxidants therapy, the data are still debatable and need further controlled evaluations in larger populations (Ashok et al., 2006).

## 6. Conclusions

The establishment of pregnancy requires a harmonic hormonal, ovarian and fallopian tube function, a receptive uterus able to respond to a variety of biochemical and molecular signals produced by the developing conceptus, as well as specific interactions between the uterine endometrium and the extra-embryonic membranes. Therefore, the fetal, placental development and function are prerequisites for an adequate supply of nutrients and oxygen to the fetus and successful establishment of pregnancy. Oxidative stress is a complex system, affecting in a complex way the female fertility, and pregnancy outcome. The imbalance of the oxidative agents and antioxidants has been proposed as the causative agents of female sterility, recurrent pregnancy loss and several pregnancy-related disorders, most notably preeclampsia, intra-uterine growth restriction (IUGR) and gestational diabetes.

Preeclampsia is characterized by increased oxidative stress due to the imbalance between lipid peroxidation and antioxidant defense mechanisms, leading to endothelial dysfunction and free radical mediated cell injury. Other maternal factors including activated neutrophils and imbalance between anticoagulants and procoagulants aggravate the oxidative stress and endothelial dysfunction this plays crucial role in development of the disease. There is no doubt that both hypoxia and hypoxia-reperfusion lead to reactive oxygen species production, but both may also arise from the same underlying problem of impaired conversion of the spiral arteries. The effects of the reduced trophoblast invasion associated with complicated pregnancies can easily be superimposed on this basic model. Reduced invasion will leave the spiral arteries vasoreactive, and thus more likely to undergo spontaneous transient vasoconstriction. They will be more responsive to endogenous and

exogenous vasoactive stimuli. Partial obliteration of their lumens by atherotic changes will also impair flow. Excessive production of inflammatory cytokines, deportation of apoptotic microvillous placental fragments, activation of maternal leukocytes and platelets, or depletion of NO production may then cause or contribute to the maternal endothelial response. The degree of the oxidative stress will likely reflect the extent of the maternal vascular pathology.

There are a number of pathways that may contribute to oxidative stress observed in the gestational diabetes mellitus placenta. In the placenta, reactive oxygen species and reactive nitrite species are an important source of growth and signaling factors, and are susceptible to ROS-mediated apoptosis. The placenta is endowed with many antioxidants, some of which are increased in gestational diabetes mellitus. However, there is much data to indicate that maternal diabetes during pregnancy may induce oxidative stress in the newborn that may entail biochemical disturbances of the fetus (Hung et al., 2006). Given that the placenta provides the interface of the maternal and fetal circulations, it may play a crucial role in protecting the fetus from adverse effects of the maternal diabetic milieu (Lappas et al., 2011).

The investigation of oxidative stress is inevitable for better understanding of aerobic organism function. Evaluation of environmental factors effect on oxidative stress molecular pathways can serve possible solutions for female reproductive malfunctions. There are several fertility and pregnancy related diseases, as unexplained infertility, preeclampsia, HELLP syndrome where the reactive oxidative species and antioxidant mechanism play key role in pathogenesis of the disease. The antioxidant supplementation, avoidance of different environmental factors, as polluted comestibles may lead to decrease of infertility rate and incidence of pregnancy related disorders.

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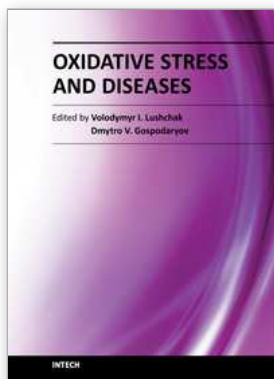
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## **Oxidative Stress and Diseases**

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The development of hypothesis of oxidative stress in the 1980s stimulated the interest of biological and biomedical sciences that extends to this day. The contributions in this book provide the reader with the knowledge accumulated to date on the involvement of reactive oxygen species in different pathologies in humans and animals. The chapters are organized into sections based on specific groups of pathologies such as cardiovascular diseases, diabetes, cancer, neuronal, hormonal, and systemic ones. A special section highlights potential of antioxidants to protect organisms against deleterious effects of reactive species. This book should appeal to many researchers, who should find its information useful for advancing their fields.

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