



An Overview of Oxidative Stress and Its Effect on Fetal Development and Organogenesis

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ABSTRACT

Oxidative stress (OS) is the state described by a disproportion of pro-oxidant and endogenous anti-oxidative defense system (ADS). In our body system, reactive oxygen species (ROS) are continuously formed as a consequence of biochemical reactions, e.g. within the mitochondrial respiratory chain and from external factors. Reactive oxidant molecules play important roles in many physiological processes like-intracellular signaling cascades to maintain cellular homeostasis with its surrounding milieu. But at higher levels, they can cause indiscriminate damage to biological molecules, leading to functional alteration and even cell death. And the scenario gets even worse in case of pregnancy when OS can lead its pathophysiologic roles in the placenta, embryo and the fetus. In this review, we will address the generation of pro-oxidants, its normal physiological role in intra-uterine environment establishment in placenta and in case of excess OS, its detrimental effects on fetal development and organogenesis.

KEYWORDS: Oxidative stress (OS), Reactive Oxygen Species (ROS), antioxidants, miscarriage, placenta

1. INTRODUCTION

Biological system holds plentiful amount of oxygen. It plays double role as it has both positive effects and potential harmful side-effects in biological system. As a diradical, oxygen readily reacts with other radicals. Thus free radicals are

often generated from oxygen itself and partially reduced species resulted from normal metabolic processes in the body. Reactive oxygen species (ROS) are prominent and potentially toxic intermediates, which are commonly involved in oxidative stress (OS) [



Oxidative stress occurs when the generation of oxidant molecules exceeds scavenging by antioxidants because of excessive production of oxidants or insufficient intake or increased utilization of antioxidants [2]. ROS usually affect the biomolecules within the immediate vicinity of its production. Different levels of OS will generate different outcomes and the clinical symptoms will therefore depend on the balance of metabolic activities in a particular cell type or organ. As a result, insignificant disturbances in the balance may lead to homeostatic adaptations in response to changes in the immediate milieu, whereas major perturbations cause irreparable damage and cell death [20].

Anti-oxidative defense system (ADS) neutralizes excessive ROS production to maintain the normal physiological functions. In female reproductive system, within the ovary and intrauterine environment, antioxidant enzymes have vital roles with protection of the oocyte and embryo from oxidative damage [40]. It has also been reported that ROS exhibit some mechanisms to affect a variety of physiologic functions (e.g. oocyte maturation, ovarian steroidogenesis, ovulation, implantation, blastocyst formation, luteolysis and luteal maintenance) during pregnancy [7, 45-48].

Over the threshold level of ROS, oxidative insult induces its deleterious effects on cellular systems including lipid peroxidation, structural and

functional modification of protein and DNA, promotes apoptosis and contributes to the risk of chronic diseases including cancer, heart disease; also in the patho-physiology of more than 200 diseases [3,4] via alteration of redox status and/or redox-sensitive signaling pathways and gene expression [5]. It is evident from in vitro, animal model and several clinical studies that OS crucially involved in the etiology of adverse reproductive events in both women and men, particularly during gestational stage of women and fetal growth period [6-13].

In addition with genetic factors, gestational environmental conditions also have great influence over fetal programming, growth trail of offspring, risk of birth defects and long-term health effects of the child [14]. This theory is known as “fetal basis of adult disease” [15], described the significance of fetal growth period when fetal genetic plasticity allows one genotype to give rise to a variety of morphological changes based on prevailing conditions. Resulting phenotypic changes are usually irreparable postnatally [14-16]. Additionally, fetal and placental development greatly relies on a precise balance of maternal intrauterine environmental conditions; disruptions of this balance may result in chronic diseases that manifest in adulthood.

Better understanding about the contribution of ROS as an influencing agent during fetal development can improve our perception to



the impact of OS on the long-term consequences leading to organ injury of programmed offspring. This review will focus on the generation of main ROS, their typical physiological role and manifestation of OS on maternal-fetal interrelationship involved in the fetal developmental stage and organogenesis during pregnancy.

2. OVERVIEW OF REACTIVE OXYGEN SPECIES

Reactive Oxygen Species (ROS) is the term for both free radicals and their non-radical intermediates. Free radicals are classified as species with unpaired valence electrons or an open electron shell which afford them having high reactivity with other molecules.

2.1 Generation of ROS

2.1.1 ROS production from basal body mechanism

Generation of ROS is occurred during biological processes of oxygen (O_2) consumption [17]. They consist of both free and non-free radical intermediates whereas the former being the most reactive. In biochemical body mechanisms, oxygen and nitrogen free radicals are recognized to be most prominent [20]. In addition, oxygen and nitrogen using biological pathways have gained greater importance because their end-products are usually found with high metabolic requirements, such as pathological processes or interaction with external environment [18].

Major ROS molecules include superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (OH^{\bullet}) and hydrogen peroxide (H_2O_2). The two common reactive nitrogen species (RNS) are nitric oxide (NO) and nitrogen dioxide (NO_2). Besides these, some non-reactive species are peroxynitrite ($ONOO^-$) and nitrosamines [19]. Other intermediates are generated by their interaction with reactive molecules.

Oxygen is used as a substrate in multiple physiological processes such as during oxygenase reactions and electron transfer reactions; these create large volume of ROS. SO anion is the most common among the oxidant molecules [21] and mitochondria are considered the principal source for its production [22].

During mitochondrial electron transport chain (ETC) reactions, some SO anions are formed as a result of leakage of electrons move towards molecular oxygen, particularly through complexes I and III due to the inefficiency of the respiratory chain enzymes. The rate of SO formation is determined by the number of electrons present on the chain. This anion is elevated under hyperoxic conditions and of raised glucose in blood. Paradoxically, hypoxic conditions also raise the amount of SO when complex IV donates the electrons to the final electron acceptor that produces reduced form of oxygen and this step causes electrons to accumulate. During normal biological condition, 2% of oxygen consumed by mitochondria is converted to SO rather than being



reduced to water. As SO is negatively charged, so it is membrane impermeable and remains within the mitochondrial matrix of the cell [20].

Additionally, outflow of electrons from the shorter electron transport chain within the endoplasmic reticulum (ER) can cause generation of superoxide anion [23]. About 25% of SO anion can be produced in ER because of the oxidative process that involves formation of disulphide bonds throughout the protein folding. This percentage of SO formation can be elevated in the cells with high secretory output and under ER stress conditions when misfolded proteins are refolded repeatedly to check the error [20].

Under physiological conditions, some superoxide producing source can be the enzymes like plasmalemmal and mitochondrial NADPH oxidases, xanthine oxidase, cytochrome P450 and by-products of numerous metabolic pathways like fatty acid oxidation [24]. Not only that, various growth factors, drugs and toxins also result increased generation of ROS [25]. So, the predominant generators of ROS are cell type specific and their formation rate is determined by metabolic energetics of that specific cell type [26, 27].

One of the major ROS is nitric oxide (NO) which is a free radical with vasodilatory properties and is an important inducer of cell signaling pathways involved in many physiological and

pathological processes [28]. It can be produced by three types of nitric oxide synthase (NOS) isoenzymes in mammals, named neuronal NO synthase (NO synthase 1), inducible NO synthase (NO synthase 2) and endothelial NO synthase (NO synthase 3) [29].

Neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS) are constitutive. Mononuclear phagocytes (monocytes and macrophages) produce a large amount of inducible NO synthase (iNOS). iNOS is expressed in response to pro-inflammatory cytokines and lipopolysaccharides [19,30,31]. During the follicular development, eNOS is expressed in granulosa cells and the surface of oocyte. In most organs, iNOS is expressed in response to immunological stimuli [32] but in pathological conditions, it might play a major role in NO production.

Over production of superoxide can lead the interaction with nitric oxide radical ($\text{NO}\bullet$) to form peroxynitrite (ONOO^-) which is a powerful pro-oxidant. It can affect neighboring cells because of its diffusion up to 5 μm [33].

2.1.2 ROS production during specific conditions

The impact of maternal lifestyle and exposure to environmental pollutants or occupational hazards [34-36] are of increasing concern because of their triggering effect to generate OS which causes abnormal pregnancy



outcomes. Studies have shown that obesity, malnutrition [37] and controllable lifestyle related choices such as cigarette smoking, alcohol use and recreational drug use [38] can act as ovo-toxicants; these may be linked to oxidative disturbances. It has been found that cigarette smoke contains a number of ROS [39] and ethanol metabolism generates ROS through the electron transport chain.

Oxidative insult can be generated through several gestational conditions also, such as prenatal hypoxia, excessive glucocorticoid exposure or an array of intrauterine conditions [40]. Hypoxia is characterized as a limited delivery of oxygen than the physiological demands of a tissue. This restrictive condition is often experienced by a growing fetus, either by maternal or umbilico-placental conditions, or by environmental hypoxia that is seen in lands with higher altitude from the sea level [41].

2.2 Detoxification of pro-oxidants

Antioxidant defenses combat with oxidative attack. Both enzymatic (e.g. MnSOD, CuZnSOD, Catalase) and non-enzymatic (e.g. Glutathione, peroxiredoxin, thio-redoxin, ascorbic acid or Vitamin C, tocopherol or Vitamin E) antioxidant [40] with antioxidant cofactors (e.g. selenium, zinc copper etc.) are capable of disposing, scavenging or suppressing the formation of ROS [42].

Two isoforms of superoxide dismutase converts superoxide to hydrogen peroxide for detoxification. The manganese isoform is restricted to the mitochondria and the copper and zinc form is located in the cytosol. Hydrogen peroxide is not a free radical, and so is less reactive than superoxide. As a non-polar molecule, it is capable of diffusing through cell and organelle membranes; acts widely as a second messenger in signal transduction pathways [20]. Catalase and glutathione peroxidase (a tetrameric selenoprotein) further degrade this product into water. Usually antioxidant enzymes act in concert because any imbalance in the concentrations of superoxide and hydrogen peroxide can result in the formation of the much more dangerous hydroxyl radical ($\text{OH}\cdot$). The hydroxyl radical has an estimated life of 10^{-9} s [43] and reacts with any biological molecule in its immediate vicinity in a diffusion-limited manner.

The enzymatic defenses have a transition metal at their core to transfer electrons during the detoxification process. Non enzymatic compounds include ceruloplasmin and transferrin also play important roles by sequestering free iron ions and inhibit the production of $\text{OH}\cdot$. Glutathione is synthesized in the cytosol and act as a major cellular thiol-redox buffer in cells [44].

Most mammalian cells contain Glutathione (GSH) and it plays an important role in cellular defense against OS by reducing protein disulfides and other cellular biomolecules. The activity of



glutathione peroxidase depends on the presence of reduced glutathione (GSH) as a hydrogen donor. GSH participates in a large number of detoxifying reactions forming glutathione disulfide that is converted back to GSH by glutathione reductase at expense of NADPH. NADPH is generated through the hexose monophosphate shunt pathway where the first enzyme is glucose-6-phosphate dehydrogenase. This enzyme is subject to common polymorphisms and reduced activity of it may decrease GSH concentrations and lead to embryopathy [44].

Vitamin E is a non-enzymatic antioxidant that tends to accumulate within the interior of lipid membranes; it is lipid-soluble in nature and holds a hydrophobic tail. There, it plays the most imperative role as a chain-breaker through reacting with lipid peroxy radicals with four times faster than they can react with neighboring fatty acid side chains. Lipid peroxidation can be proficiently detoxified in cellular system by the glutathione S-transferase group of enzymes [20].

Successful implication of small synthetic molecules that mimic antioxidant enzymes are the new tools being developed for therapeutic purposes [28]. From different experimental studies, indirect evidences have been found that antioxidant intake can get better reproductive health. Studies showed that multivitamin supplementation prior conception may improve fertility, possibly by

escalating menstrual cycle regularity or via prevention of ovulatory disorders [42].

From the ancient times, essentiality of vitamin E for fertility is well known and from the observation in rodents resulted in its dietary components being coined as 'tocopherol (propagate childbirth)' [42]. Thomas and Cheng (1952) first reported that deficiency of vitamin E can be a causative agent of congenital abnormalities. Dorothy (1964) showed in her experiment, many non-tocopherol antioxidants also can act like tocopherol in preventing and improving deficiency symptoms when fed to vitamin E deficiency animals. In this experiment, three of such antioxidants named N, N'-diphenyl-p-phenylenediamine (DPPD), N-propyl gallate (NPG) and 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EMQ) were used for their protecting role against congenital malformations in vitamin E deficient rats. It was found that all these antioxidants significantly prevented the congenital anomalies through promoting the production of normal fetus [195].

3. RELATIONSHIP BETWEEN ROS AND ANTIOXIDANTS IN THE OVARY

There is a delicate balance between ROS and antioxidants in the ovarian tissues. Sufficient proof can be found from different studies to hypothesize that dietary antioxidants and oxidative stress (OS) can influence the specific timing and maintenance of embryo implantation and viable pregnancy.



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Evidence from studies of men indicates that dietary antioxidants appear as a crucial element in preventing oxidative damage to sperm DNA [49]. Sperm-related dysfunctions associated with ROS can result reduced sperm count with motility and inhibition of sperm-oocyte fusion [6]. The female ovary is the source of oocytes and regulation of hormones and OS within gynecologic environment is probably a key mediator of conception.

During puberty, following hormonal influence, a number of primary oocytes begin to grow each month. One primary oocyte which is the dominant oocyte outgrows others and continues meiosis I (MI). Notably, resumption of MI is induced by an increase of ROS and repressed by antioxidants [50-52], representing the importance of the generation of ROS by the pre-ovulatory follicle as the central promoter of the ovulatory consequence. However, there is evidence that over time, excess ROS production can contribute to develop oophoritis related with autoimmune premature ovarian failure [52], which is worsened by diminished antioxidant status.

Oocyte maturation occurs with the second meiotic division (MII), which arises with pre-ovulatory luteinizing hormone (LH) surge [53]. Increase in steroid hormone production in the growing follicle leads an increase in P450 that causes ROS production. Pregnancy itself may produce OS as a result of increased metabolic activity. The process of MII is suspended in

metaphase and resume after fertilization following ovulation of the mature oocyte. Succession of meiosis II is continued by antioxidants produced by pre-ovulatory follicle and it is considered as an important inducer for ovulation [37]. It is found that, both in human and rat, granulosa and luteal cells respond negatively to ROS and have adverse effect on MII progression, leading to diminished gonadotrophin and anti-steroidogenic actions, DNA damage and impaired ATP generation [52].

Additionally, oxygen depletion stimulates follicular angiogenesis that is important for proper growth and development of the ovarian follicle. Follicular ROS promotes apoptosis, whereas GSH and follicular stimulating hormone (FSH) counterbalance this action in the growing follicle as estrogen increases in response to FSH and trigger generation of catalase in the dominant follicle; thus avoiding apoptosis to survive this follicle. During normal pregnancy, leukocyte activation causes inflammatory response for the increased production of superoxide anions in the 1st trimester of pregnancy [54, 55]. Notably, OS during the 2nd trimester of gestation is considered as a normal occurrence, which is supported by mitochondrial production of lipid peroxides, free radicals, and vitamin E in the placenta that increases as gestation progresses [56-58]. There are contrasting effects of antioxidants for meiosis where detrimental effect for the progression of MI but beneficial for MII



suggests a multifaceted role of antioxidants and ROS in the ovarian environment.

It has also been identified that cellular antioxidant Glutathione (GSH) has critical role in oocyte, predominantly in cytoplasmic maturation required for the formation of male sperm pronucleus and development of ovary for implantation [59]. In bovine models, beta-carotene has been reported to enhance cytoplasmic maturation, further supporting reports also found in other species [60].

Successful initiation of pregnancy requires a successful chain of events such as the ovulation of a mature oocyte, production of competent sperm, proximity of sperm and oocyte in the female reproductive tract, oocyte fertilization, and transport of the conceptus into the uterus and implantation of the embryo into a properly prepared and healthy endometrium. Dysfunction in any one of these complex biological steps can cause pregnancy related complications [61].

4. PLACENTA: THE DEVELOPMENTAL PLACE OF FETUS

Placenta is a fundamental organ of pregnancy to serve as a maternal-fetal connection and functions as an immunologic and nutritional intermediary between mother and child. It produces growth hormones, cytokines and signaling molecules to drive placental and fetal development [22, 62].

The human placenta has unique process of formation because villi form initially over the entire surface of the chorionic sac [20]. The placental vasculature undergoes changes to ensure optimal maternal vascular perfusion. Prior to the unplugging of the maternal spiral arteries, low O₂ tension in early gestational stage results a normal, physiological hypoxia [63]. Maternal arterial blood is prevented from invading to the intervillous space of the placenta by plugs of extravillous cytotrophoblast cells that occupy down the mouths of the uterine spiral arteries [64, 65]. Onset of maternal circulation is associated with a three-fold rise in oxygen concentration within the placenta [66] and this process is linked with an increase in ROS, which leads to OS. The maternal intra-placental circulation is only fully established towards the end of the first trimester (between 10 and 12 weeks of gestation), the trophoblastic plugs are dislodged from the maternal spiral arteries and flood the intervillous space with maternal blood. From the end of the first trimester, the villi over the superficial pole regress to leave the ultimate discoid placental form. It is now evident that OS plays a central role in this process and this regression is considered as normal physiological changes during pregnancy [20].

As embryo has low antioxidant capacity and is highly sensitive to injury by the oxidant molecules and oxidant free radical mediated teratogenesis [67], so development of embryo occurs in relatively low oxygen environment [68,



69] and it is supported by the secretion from the maternal endometrial glands rather than the maternal circulation [66, 70]. But increase oxygen transfer towards developing fetus is occurred in placental maturation for sustaining the increased metabolic rate during the rapid fetal growth phase [71]. This increased supply of oxygen elevates the cellular production of ROS [72] which initiates a switch from reduced to oxidized form in the cellular redox state to stimulate cellular differentiation [70, 73] particularly in the critical syncytiotrophoblastic layer, which contains low concentrations of the principal antioxidant enzymatic defenses, copper and zinc superoxide dismutase and catalase [74,75]. At physiological concentrations, ROS serve as signaling molecules to induce transcription of several genes (e.g. *HIF1A*, *CREB1*, *NFKB1*) that are important for oxygen sensing, cell differentiation and proliferation [73, 74,76].

Placental acclimation to increased O_2 tension up-regulates antioxidant gene expression and activity to protect fetal tissue against the deleterious effects of ROS during the critical phases of embryogenesis and organogenesis [77]. If maternal blood flow reaches the intervillous space prematurely, placental OS can proceed too early to cause deterioration of the syncytiotrophoblast.

Any abnormal incidents associated reactive oxidants before conception, during implantation and maturation of embryo can give rise to a variety of complications to both mother and fetus [68].

5. EFFECT OF ROS ON FETAL GROWTH

Our body is under continuous oxidative attack from different reactive oxidant molecules. Oxygen toxicity can have multiple and diverse effects on a cell and it is best defined in broad terms as an alteration in the pro-oxidant-antioxidant balance where pro-oxidant concentration is higher that causes potential cellular damage [78]. Animal studies support the hypothesis that OS induces programmed phenotypes in the adult offspring. OS is now recognized as a key agent in association with pregnancy related complications and may be a contributing factor in the pathophysiology of different disorders of fetus and neonates. For example: excessive OS modifies lipids, proteins and DNA leading to placental endothelial dysfunction, ischemia-reperfusion injury, interrupted nutrient (calcium) transfer to fetus, altered osteogenic gene transcription and fetal maldevelopment of functional organs [14, 79-84].

5.1 Deleterious effect of ROS on cellular system

The outcome of OS depends upon the cellular compartment in which the ROS are generated. It has been observed that moderate increase in ROS levels can stimulate normal physiological functions like cell growth and proliferation. In the contrary, excessive ROS will cause cellular injury (e.g., structural and functional alteration of protein and DNA, oxidation of amino acids and lipid membranes). Increased level of SO



anion and H_2O_2 may generate a more toxic hydroxyl radical $\text{OH}\cdot$ which modifies purines and pyrimidines that causes DNA strand breakage and DNA damage [85]. Excessive production of RNS can alter protein structure and function, thus can result changes in catalytic activity of enzyme, cytoskeletal organization and impaired cell signal transduction [21,17].

Mitochondria is the power house of metabolic activities in cells, so even minor interruption in their functions can lead to extremely altered generation of adenine triphosphate (ATP). Additionally, they are principal targets of OS because the mitochondrial genome lacks histones and has minimal DNA repair mechanisms than the nucleus [86]. Mitochondrial protein and membrane damage can lead to promote further mitochondrial dysfunction. This assault may result in a positive feedback mechanism in such a way that failure of only a few mitochondria that are injured by OS can direct an entire network of mitochondria to fail [87].

During pregnancy, energy from ATP is essential for gamete functions therefore, excessive ROS can affect functions of the mitochondria in oocytes and embryos [88, 89]. The enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase generates significant quantities of superoxide throughout pregnancy, particularly in early gestational age [90]. Thus, fetal organs that are highly dependent on oxidative phosphorylation

and β -fatty acid oxidation may be more vulnerable to oxidative stress both in utero and after birth [40].

One of the most challenging conditions during fetal development is hypoxic condition raised in intrauterine environment. One of the mechanisms by which hypoxia induces damage is the increased generation of ROS [91-93]. Again hypoxia of blood may provoke increased ROS generation in the mitochondria of endothelial and placental cells [94]. This is considered harmful to the fetus for its short and long-term distressing effects, particularly in the central nervous and cardiovascular systems resulting in functional alterations. Additionally, in utero undesirable conditions can lead to develop chronic diseases later in life; a phenomena known as fetal programming or developmental origins of health and disease [95-97].

5.2 Spontaneous abortion

Spontaneous abortion refers to the unintentional termination of a pregnancy before fetal viability at 20 weeks of gestation or when fetal weight is < 500 g. Recent studies have shown that 8% to 20% of clinical pregnancies become aborted due to spontaneous miscarriage before 20 weeks of gestation [28].

Vast placental OS has been proposed as a causative factor of spontaneous abortion. Beginning of the maternal circulation, placentas of normal pregnancies experience an oxidative burst between



10 and 12 weeks of gestation [28], which resolves the placental tissues to adapt to their new oxygen environment. In cases of abortion, the onset of maternal intra-placental circulation is both precocious and disorganized compared with normal ongoing pregnancies [67,98] and it occurs prematurely between 8 and 9 weeks of gestation [99, 100]. Antioxidant enzymes are unable to counter the augment of ROS at this point as their expression and activity increases with gestational age [99].

Also, when OS develops too early in pregnancy it can impair placental development. Levels of HSP70 and nitrotyrosine are increased in the villi of central region and the periphery of these placentas [98, 101]. The apoptotic index is also high compared with control placentas of a similar gestational age and morphological evidence has clearly shown the degeneration of syncytiotrophoblast. Therefore, overwhelming OS causes widespread destruction of the trophoblast that is incompatible with an ongoing pregnancy [67].

In these cases, the efficacy of the antioxidant defenses provides detoxification of ROS but polymorphisms in these enzymes occur in some instances that have been linked to an increased risk of miscarriage [102-104]. Equally, some evidence shows that selenium deficiency, which will reduce the efficacy of glutathione peroxidase, is associated with miscarriage [105, 106].

5.3 Intrauterine growth restriction and fetal development

Intrauterine growth restriction (IUGR) is defined as the infant birth weight below 10th percentile [107]. Nowadays, it is estimated that worldwide there are 150 million new born per year and 5–10% of them have low birth weight standardized for gestational age and increases the risk for peri-natal morbidity and mortality [108, 109].

Placental, maternal and fetal factors are the most common causes of IUGR. Placental insufficiency can lead to IUGR due to a decreased fetoplacental perfusion and restricted oxygen supply. Ischemia and reperfusion injury are powerful generators of ROS and OS. Therefore, OS is recognized as an important player in the development of IUGR [110].

From different studies, it has been found that women with IUGR have increased free radical activity and markers of lipid peroxidation in their blood [111]. Biri et al (2007) reported increased levels of Malondialdehyde (MDA) and xanthine oxidase, urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OxOdG), a marker of DNA oxidation and lower levels of antioxidant concentrations in the plasma, placenta and umbilical cords in patients with IUGR compared to controls at 12 and 28 weeks in pregnancies [110, 112]. Moreover, the impact of intrauterine stress on the affected offspring is influenced by the severity



and duration of the insult, as well as, the gestational age of fetal exposure [113, 114].

Epidemiological evidence and animal model studies have identified a clear association between low birth weight and an increased incidence of different diseases like hypertension, type II diabetes, metabolic syndrome, insulin resistance and obesity [113-116]. There is an inverse relationship between birth weight and mortality rate due to cardiovascular disease [117-119] and systolic blood pressure [120]. The role of OS in fetal programming is supported by epidemiological evidence of oxidant indices and low birth weight in association with type 2 diabetes [121], cardiovascular disease [122] and preeclampsia [123]. Thus, OS may be a connecting link between IUGR and fetal programming consequences after birth.

5.4 Effect of ROS on fetal heart function

Recent animal studies have identified an important role of oxidant molecules in normal physiological function of the fetal cardiovascular system. Antioxidant administration of melatonin or vitamin C to pregnant sheep increased umbilical vascular [124] and fetal femoral artery conductance [125] respectively through the mechanisms of increased nitric oxide (NO) bioavailability following decreased superoxide anion levels. In addition, inhibition of superoxide anion attenuated the fetal sheep pressor responses and increase umbilical

vascular conductance by altering NO bioavailability and activation of β_1 adrenoceptor mechanisms respectively [126]. Thus, oxidant molecules play an important physiological role in maintaining fetal cardiovascular homeostasis.

It has also been reported that ROS provoke relaxation of ductus arteriosus by stimulating prostaglandin synthesis [127]. Hydrogen peroxide has a role to exert a dose-dependent biphasic effects on human heart, provoking an increase of contractile force followed by a decrease [128]. In another study, H_2O_2 was observed to stimulate bradycardia and hypotension in rats [129]. Therefore, increased blood level of H_2O_2 and superoxide can be accountable for abnormalities of heart rate.

Prenatal hypoxia has been shown to generate OS in fetal hearts in a variety of animal species, such as, sheep [130], rat [102, 131] and guinea pig [132-134]. Hypoxic condition increases the expression of fetal cardiac inducible NO synthase [132, 134], nitrotyrosine [102, 133], heat shock protein 70 (HSP70) [102, 135], proinflammatory cytokines [136] and matrix metalloproteinases [134, 136-138]; suggesting the generation of oxidative and inflammatory stress as causative agent. Prenatal treatment with the antioxidant (N-acetylcysteine) inhibits the peroxynitrite levels and fibrosis in fetal guinea pig heart ventricles [133]. Prenatal vitamin C inhibits fetal cardiac HSP70 expression and adult



myocardial contractility associated with β_1 adrenoceptor stimulation in rat offspring [102]. Moreover, neonatal rats with increased ROS production is associated with elevated systolic blood pressure and vascular dysfunction in adults [139].

Taken together, these studies suggest that oxidative stress is an important stressor that impacts on fetal heart rate and instigates cardiovascular programming of the offspring but imbalance of ROS can affect normal fetal cardiac output [40].

5.5 Effect of ROS on fetal liver and kidney function

Oxidative stress in hepatic tissues of fetus can make the adult liver more susceptible to non-alcohol associated fatty liver diseases [140]. OS has also effect on fetal kidney function. The kidney plays an important role in programmed hypertension when exposed to an intrauterine environment that is altered by OS. Offspring that developed in a high-oxidative stress environment exhibited elevated expression of some proteins: peroxiredoxin, HSPB6, SOD-1 and PPAR γ in kidney when compared to their counterparts who developed in a normal intrauterine environment, identifying a role of oxidative stress in contributing to developmental programming of kidney-associated hypertension [40].

5.6 Fetal oxidative stress and diabetic embryopathy

Reactive oxidant molecules are involved in the etiology of numerous diseases including diabetes mellitus [141]. It has been observed that pancreatic β cells of offspring of IUGR fetuses are vulnerable to OS because of its relatively weak antioxidant defense and impaired ATP generation [142] especially at the early stages of organogenesis, result in severe embryonic damage.

The proportion of congenital anomalous neonates of diabetic mothers is much higher than that of normal neonatal population. Congenital malformations that are occurred before early gestational period (7th week of gestation) results various central nervous system deformities such as anencephaly, spina bifida, and hydrocephalus; and cardiac anomalies. Hypothesis that ROS plays a vital role in this pathophysiology of embryonic dysmorphogenesis in diabetic mother is based on the protective role of antioxidative defense enzymes against excess glucose and other oxidative substrates. It has been found that GSH regeneration by GSSG conversion which needs NADPH, becomes deficient here for the maintenance of intracellular GSH during organogenesis under diabetic conditions [194]. Ornoy (2007) had done an experiment to evaluate the role of OS in diabetic induced embryopathy, both in vivo and in vitro. It has been shown that under diabetic condition, there was a significant decrease in the activity of



endogenous antioxidant enzymes such as vitamins C and E in the embryos and yolk sacs. The lowest activity was observed in the malformed experimental embryos compared to experimental embryos without anomalies. Similar results were obtained in the cohen diabetic rats (CDR), where these diabetic prone rats were unable to increase their antioxidant enzyme activity in spite of the diabetes [141].

5.7 Interrelationship of OS and obesity on fetal growth

Obesity is one of the most burdensome public health concerns in modern time. The firm increase in overweight reproductive-age women is correlated with the increase of childhood and infant obesity. A possible link between the abnormal intrauterine environment and abnormal growth of offspring must be considered [143]. It has been observed that obesity in the non-pregnant and pregnant state is associated with inflammation and OS [144-157]. The period from conception to birth is a time of rapid growth, cellular replication, differentiation and functional maturation of organ systems. These processes are very sensitive to alterations of intrauterine metabolic milieu during pregnancy which can have long-term effects on the development of obesity and diabetes in offspring [144, 145].

Several investigators have used animal models of high-fat or Western style diet-induced obesity (a diet that has increased fat and

carbohydrate content) during pregnancy increases OS, thereby potentiating adipogenesis in the offspring [158-171]. Offspring from dams fed the Western diet had significantly increased adiposity as early as 2 weeks of age as well as impaired glucose tolerance compared with offspring of dams fed a control diet. Inflammation and OS were increased in pre-implantation embryos, fetuses and newborns of Western diet-fed rats. Gene expression of proadipogenic and lipogenic genes was altered in fat tissue of rats at 2 weeks and 2 months of age. But the addition of an antioxidant supplement decreased adiposity and normalized glucose tolerance [172].

5.8 Embryonic oxidative stress and fetal distress

Fetal distress is collectively known as a pathophysiological condition in which oxygen is not available to the fetus in sufficient quantities [173]. If not corrected or circumvented properly, it may cause aberration of physiological responses or even result in multiple organ damage [174, 175]. Fetal distress could be associated with OS in fetal and maternal blood [176, 177]. Raičević et al. (2010) had done an experiment to determine the relation between fetal distress and OS to explore the application of specific biochemical assays of oxidative status parameters for fetal distress prediction. Here, oxidative status was evaluated by measuring the levels of superoxide and hydrogen peroxide in plasma and the activities of



corresponding antioxidative enzymes- superoxide dismutase and catalase in erythrocytes [178].

The result found that oxidative stress is predominantly of fetal origin as the concentration of superoxide was increased only in the fetal plasma. Superoxide is converted to H_2O_2 which can pass from the fetus into the maternal blood and vice versa, resulting in similar H_2O_2 concentrations in fetal and maternal placental tissue, which also support the previously established good correlation between maternal and fetal oxidative statuses [179].

5.9 Oxidative stress and fetal bone formation

Neonatal bone mass and osteogenesis are positively correlated with placental weight [63, 180]. Fetal bone is delicately sensitive to environmental influences especially in mid-gestation, when the appendicular skeleton rapidly grows in all dimensions and in volumetric density. Adverse exposures which may produce OS can alter trail of fetal osteogenic regulation in an approach that increases risk of adult bone dysfunction [181].

Pilot mouse studies had done by Prater et al. (2007) on C57BL/6 mice. Here, teratogenic alkylating agent methylnitrosourea (MNU) was administrated to induce OS-mediated fetal and placental pathology [182]. Human mainly expose to MNU primarily from two sources: tobacco smoke and endogenous metabolism of nitrosated foods

[183]. MNU was administered in mice with and without dietary antioxidant quercetin (Q) supplementation and it was experimentally administered at gestation day 9 (GD9) in mice when limb bud formation and rapid placental development is occurred [182, 184-187]. Several key placental proteins that influence placental development and fetal osteogenesis (Hgf, Kitl, $IFN\alpha 4$, Ifrd, and $IL-1\beta$) were altered by MNU, resulted in small fetuses with disproportionately shortened limbs and distal limb malformations and caused damage of placental endothelium and trophoblast. Q was shown protective against these fetal and placental pathologies [15,180,188].

Antioxidant properties of quercetin increases osteogenic differentiation of MSC, elevates diaphyseal calcium, enhances bone tensile strength, upregulates bone matrix formation and increases trabecular bone density. These beneficial effects of Q are thought to collectively reduce incidence and symptoms of osteoporosis during fetal bone formation [189-193].

6. CONCLUSION

The effect of OS in the development of fetus is an important area deserving of continued research. The available evidence suggests gynecologic OS is an important mediator of conception and threshold levels that depend on anatomic location and stage of preconception



ensure for the benefit or harm of the developing embryos.

Exposure to different teratogens, drugs, alcohol and environmental pollution can also give rise to excessive OS during pregnancy and has increasingly raised concern about the impact of OS on maternal and fetal health. Fetal organ-specific responses are dependent on the relative balance between ROS generation and the antioxidant capacity of that cell. Identification of gene targets vulnerable to OS is also important for understanding the cell-specific responses to intrauterine stress, as well as, developing

therapeutic strategies for alleviating long-term programmed consequences of fetus associated with adult disease.

Human research investigating dietary antioxidants to compensate OS can be especially challenging because in vitro studies of humans and other mammals offer promising insight, but in vivo condition is not fully known yet. In the future, human clinical trials will help to clarify the efficacy of antioxidants as potential therapies for the better outcomes to solve the pregnancy related complications and proper fetal organogenesis and development during that time.

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