Oxidative Stress in Applied Basic Research and Clinical Practice

Ashok Agarwal
Nabil Aziz
Botros Rizk *Editors*

Studies on Women's Health

Humana Press

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To my father Professor RC Aggarwal for instilling the virtues of honesty, dedication, and hard work. To Professor CJ Dominic (late) (Banaras Hindu University), Professor Kevin Loughlin (Harvard Medical School), and Dr. Anthony Thomas (Cleveland Clinic) for making an indelible positive impression on my life

Ashok Agarwal

I dedicate our book to my dear father, Mitry Botros Rizk—a philosopher and thinker—for his inspiration, my mother, Dr. Isis Mahrous Rofail—a marvelous clinician and surgeon—for her unlimited love and sacrifice, my dear father-in-law, Dr. George Nawar Moussa—an accomplished scientist—for his spiritual guidance, and to my mother-in-law, Aida Erian Attalla—a talented artist—for her genuine care and sincerity

Botros Rizk

I would like to dedicate this book to my father Professor Fahim Aziz and my mother who inspired me to peruse excellence remembering humility at all times. I would like also to dedicate it to my wife Howayda and my daughters Sally and Amy for their continued support

Nabil Aziz

Foreword

Books can be separated into two categories: those that are exploratory and forward looking, that propose to define new pathways and to "change the way we think about a topic" and those that are exploratory and forward looking. This book is a good blend of both types. Its subject matter is oxidative stress in women health. Oxidative stress and how to prevent its impact on human diseases has become a hot scientific and clinical topic over the last decade. There is mounting evidence that oxidative stress or an imbalance in the oxidant/antioxidant activity in female organs and tissues plays a pivotal role in the development of infertility, cancer, and placental-related diseases of pregnancy.

Evolution for mammals living on dry land has been closely linked to adaptation to changes in O_2 concentration in the environment and thus it is not surprising that the oxygen metabolism by human tissues has such an impact on human reproduction. Basic science studies have shown that most of the O_2 used during the oxidation of dietary organic molecules is converted into water via the combined action of the enzymes of the respiratory chain. Around 1–2 % of the O_2 consumed escapes this process and is diverted into highly reactive oxygen free radicals (OFR) and other reactive oxygen species (ROS) at a rate dependent on the prevailing oxygen tension. When the production of OFR exceeds the cellular natural protection, indiscriminate damage can occur to proteins, lipids, and DNA. The consequences may range from the activation of stress-response proteins through to apoptosis or necrosis.

Human reproduction and early fetal development in utero is influenced and modulated by a constant adaptation to ambient oxygen concentrations. Oxidative stress influences the entire reproductive life span of a woman and beyond during the menopause phase of her life. In human reproduction it influences the oocyte and embryo quality and thus the fertilization rates. ROS appears to play a significant role in the modulation of gamete interaction and also for successful fertilization to take place. Oxidative stress exacerbates the development of endometriosis by inducing chemoattractants and endometrial cell growth-promoting activity and the oxidative proinflammatory state of the peritoneal fluid is an important mediator of endometriosis. Thus oxidative stress affects multiple

physiological processes from oocyte maturation to fertilization, embryo development and pregnancy but there is also new evidence that free radicals in small concentrations are essential for normal cellular function and modulate the activity of many transcription factors.

There is increasing evidence indicating that failure of placentation leading to a specific human pregnancy disease such as preeclampsia is associated with an imbalance of free radicals, which will further affect placental development and function and may subsequently have an influence on both the fetus and its mother. Maternal metabolic disorders, for example diabetes, and lifestyle factors such as alcohol consumption and cigarette smoking, which are associated with an increased production of free radicals species, are also known to be associated with a higher incidence of miscarriage and fetal structural defects. Furthermore, the teratogenicity of drugs such as thalidomide has recently been shown to involve free radical-mediated oxidative damage, indicating that the human fetus can be irreversibly damaged by oxidative stress.

The chapters in this book cover all the important aspects of the relationship between oxidative stress and women health and the editors have to be congratulated on their vision and the way in which this book provides a comprehensive review of this important topic.

Eric Jauniaux

Preface

God created man in excellent health. While there is extensive documentation on life and death in the ancient civilizations, there is scarce literature on when man started suffering from disease. (1) The ancient Egyptians were the first to extensively document medical interventions. The ancient Egyptians left for us impressive illustrations of neurosurgery, childbirth, and circumcision. Some of these date back to 2600 BC. (2) We also have historical documentation of infertility in the Old Testament related to events between 2000 and 1700 BC that are very well known in the history of the Patriarchs, Abraham, Isaac, and Jacob.

Most medical books focus on a medical problem or symptom and then attempt to discuss the different medical evaluations and their medical and surgical treatments. Our book on oxidative stress and women's health is an exception. This book focuses on the involvement of a medical issue, oxidative stress, in many pathological situations and diseases. At the beginning, the editors worked diligently to find the concept that ties all these very different pathologies. The book is the first comprehensive book that explores the involvement of oxidative stress in oogenesis, pregnancy, placental functions, as well as endometriosis and cardiovascular disease. While some of these issues such as endometriosis are easier to understand, others, we must admit, await further investigations. Our book is the beginning of the exploration of a new field and in a few years, many of the discussed topics will be totally redefined but we take great pride in presenting the first idea. To our dear readers, we put in your hands a novel book that we hope that will stimulate your scientific curiosity and interest and prove to be an enjoyable reading experience.

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Chapter 1
Maternal Nutrition, Oxidative Stress and Prenatal Devlopmental Outcomes

Kaïs Hussain Al-Gubory

Abstract Aerobic organisms have adapted themselves to a coexistence with reactive oxygen species (ROS) by developing various and interdependent antioxidant systems that includes enzymatic and non-enzymatic antioxidants. Dietary antioxidants also play important roles in protecting the developing organisms from ROS damage, and both dietary and enzymatic antioxidants are components of interrelated systems that interact with each other to control ROS production. Oxidative stress can arise from an imbalance between generation and elimination of ROS leading to excessive ROS levels that damage all biomolecules. Tightly controlled ROS generation is one of the central elements in the mechanisms of cellular signaling and maintenance of signal transduction pathways involved in cell function, growth, and differentiation. Oxidative stress is considered to be a promoter of several prenatal developmental disorders and complications, importantly defective embryogenesis, embryopathies, embryonic mortality, spontaneous abortion, recurrent pregnancy loss, fetal growth restriction, intrauterine fetal death, low birth weight, preeclampsia, and preterm delivery. Environmental chemicals in food, water, and beverage may contribute to such adverse prenatal developmental outcomes and increase the susceptibility of offspring to disease via impairment of the antioxidant defense systems and enhancement of ROS generation. This chapter deals with the state of knowledge on the association between ROS, oxidative stress, antioxidants, and prenatal developmental outcomes. The importance of maternal antioxidant-rich foods in eliciting favorable effects on women health and prenatal development outcomes is highlighted.

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1.1 Introduction

Reactive oxygen species (ROS) are molecules and free radicals produced during metabolic pathway that uses energy released by the oxidation of nutrients to produce adenosine triphosphate (ATP). The concept of the paradox of aerobic life [1] or the paradox of ROS [2] has been emerged for our acceptance that life of aerobic organisms is a paradox. Tightly controlled ROS generation is an important constitutive process and is one of the central elements in cell signaling and maintenance of redox homeostasis [3] and signal transduction pathways involved in cell function, growth, differentiation, or death [4–6]. To maintain redox homeostasis and to cope with injury from ROS-induced oxidative damage, aerobic organisms have adapted to a coexistence with ROS through the development of various and interdependent antioxidant systems that includes enzymatic and non-enzymatic antioxidants [7]. Various dietary antioxidants also play important roles in protecting cells from ROS damage, and both dietary and enzymatic antioxidants are components of interrelated systems that interact synergically with each other to control ROS production. The balance between ROS and antioxidant systems determines the degree of oxidative damage to biological macromolecules. Abnormally high ROS generation leads to irreversible alteration of biomacromolecules, mainly lipids, proteins, and nucleic acids, ultimately causes mitochondrial dysfunction, mitochondrial ROS-induced ROS release (RIRR), and cell death by apoptosis [8].

ROS and antioxidants cross-talk is important component of the mammalian reproductive functions, such as ovarian follicular development, luteal steroidogenesis, endometrium receptivity, embryonic development, implantation, placental development, and growth [9, 10]. Maintaining equilibrium between antioxidants and ROS is crucial for the survival of the developing organisms. High levels of ROS during embryonic, fetal, and placental development are a feature of pregnancy [11, 12]. ROS-induced oxidative stress has emerged as a likely promoter of several prenatal developmental disorders and complications, such as defective embryogenesis, embryopathies, embryonic mortality, abortion, idiopathic recurrent pregnancy loss, hydatidiform mole, fetal growth restriction, intrauterine fetal death, low birth weight, preeclampsia, and preterm delivery [13–15]. Maternal malnutrition and/or maternal exposure to environmental chemicals may contribute to such adverse prenatal developmental outcomes and increase the susceptibility of offspring to disease. This occurs, at least in part, via impairment of the antioxidant systems and enhancement of ROS generation which alters cellular signaling and/or

damage biomacromolecules. The links among ROS, oxidative stress, antioxidants, the female reproductive system, and adverse prenatal developmental outcomes, constitute therefore important issues in human and animal reproductive medicine.

Maternal nutrition is one of the most important lifestyle factors determining embryonic/fetal development. Malnutrition plays major roles in programing the offspring susceptibility to oxidative stress and disease [16]. Man-made chemicals are of increasing public health concern owing to an increasing number of such environmental pollutants arising from industrial and agricultural activities. Prenatal exposure to various environmental chemicals occurs through the consumption of contaminated food, water, and beverage. Maternal exposure to environmental chemicals during the critical periods of pregnancy [17], pass across the placental barrier into the fetal blood, transferred to the fetus, and may affect developmental outcomes [18]. This chapter deals with the state of knowledge on the association between oxidative stress, antioxidants, and prenatal developmental outcomes. In recent years, antioxidant nutritional interventions will provide sensible strategies against maternal malnutrition and/or environmental chemical insults. The importance of antioxidant-rich foods in eliciting favorable effects on women health and prenatal development outcomes is highlighted.

1.2 Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) includes oxygen free radicals, such as the superoxide anion radical ($^{\cdot}O_2^-$), hydroxyl radical ($^{\cdot}OH$), and nitric oxide ($NO^{\cdot}$), and also non-radical reactive oxygen derivatives, such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$). A free radical is a chemical species containing one or more unpaired electrons in its outer orbital. The ROS are ubiquitous, highly reactive, and diffusible molecules. ROS may promote oxidative damage to proteins, lipids, and DNA when they are overabundant. The consequences of these attacks are respectively, loss of enzymes activity, cell membrane alterations, DNA lesions, and oxidative mutagenesis. The generation of $^{\cdot}O_2^-$ by a single electron donation to dioxygen molecule (O_2) is the first step in the formation and propagation of ROS within and out of the cell. The toxicity $^{\cdot}O_2^-$ is based on generation of very reactive ROS, so-called downstream products of $^{\cdot}O_2^-$. The $^{\cdot}O_2^-$ radical is the precursor of most ROS and could be a mediator in oxidative chain reactions. Dismutation of $^{\cdot}O_2^-$ produces H_2O_2 and O_2 in accordance with McCord and Fridovich [19] reaction:

$$^{\cdot}O_2^- + ^{\cdot}O_2^- + 2H+ \rightarrow H_2O_2 + O_2$$

In the presence of free unbound iron and/or copper ions, H_2O_2 interact with $^{\cdot}O_2^-$ in a Haber–Weiss reaction [20] which is considered as a common source of the highly reactive and oxidative $^{\cdot}OH$. The Haber–Weiss cycle consists of the following reactions:

$$Fe^{3+} + {}^{\cdot}O_2^- \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH \,(\text{Fenton reaction})$$
$$Cu^{2+} + {}^{\cdot}O_2^- \rightarrow Cu^{+} + O_2$$
$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^- + {}^{\cdot}OH$$

The net reaction:

$${}^{\cdot}O_2^- + H_2O_2 \rightarrow {}^{\cdot}OH + OH^- + O_2$$

The resulting Fe^{2+} could propagate the oxidative degradation to new amino acid residues by reacting with H_2O_2 to form further $^{\cdot}OH$. As a consequence of such reactions, uncontrolled generation of highly reactive ROS can lead to oxidative damage to cellular biomolecules and cell death by apoptosis [21, 22]. Since there is no known pathway to remove $^{\cdot}OH$, the generation of $^{\cdot}OH$ from $^{\cdot}O_2^-$ and H_2O_2 is considered the central mechanism by which H_2O_2 induces damage to DNA, proteins, and lipids in biological systems [7].

Other important cellular ROS messenger is $NO^{\cdot}$. It is produced from L-arginine in a reaction catalyzed by NO synthase (NOS) and acts as a regulator of many physiologic events [23]. NO is also an important prooxidant and toxic factor. Prooxidant action of $NO^{\cdot}$ is often attributed to NO-derived species such as $ONOO^-$ which possess strong oxidative properties. Indeed, $NO^{\cdot}$ may react with $^{\cdot}O_2^-$ in a reaction controlled by the rate of diffusion of both radicals to form $ONOO^-$ [24] as follows:

$$NO^{\cdot} + {}^{\cdot}O_2^- \rightarrow ONOO^-$$

The molecule $ONOO^-$ can diffuse freely within and out of the cell, and react with lipids, proteins, and DNA, leading to cell membrane lipid peroxidation [25], DNA damage, and apoptosis [26]. Importantly, protein S-nitrosylation inhibits the activity of enzymes known to be S-nitrosylated by $NO^{\cdot}$ [27], such as caspase-3 [28]. Decrease in caspase-3 S-nitrosylation is associated with an increase in caspase activity [29]. It is important to highlight that the balance between $NO^{\cdot}$ and $^{\cdot}O_2^-$ and consequent $ONOO^-$ production determine the degree of cellular oxidative damage [30].

1.3 Cellular Sources of Reactive Oxygen Species (ROS)

The main site of ROS production, mainly $^{\cdot}O_2^-$, H_2O_2, and $^{\cdot}OH$, is the mitochondrial electron transfer chain. The machinery of mitochondrial oxidative metabolism and ATP synthesis is associated with the inevitable ROS production and propagation within and out of the mitochondria. The inner membrane mitochondrial respiratory chain consists of four multimeric complexes (complexes I-IV), coenzyme Q (CoQ), and cytochrome C (Cyt C). The production of $^{\cdot}O_2^-$ occurs during the

passage of electrons through the mitochondrial electron transport system, so-called respiratory chain oxidative phosphorylation (OXPHOS). This process is the major ATP synthetic pathway in eukaryotes [31]. It comprises the electron transport chain that establishes a proton gradient across the mitochondrial inner membrane by oxidizing the reduced nicotinamide adenine dinucleotide (NADH) produced from the Krebs cycle. The main substrate for NADH is supplied by cellular glucose, fatty acids, and amino acids via three interconnected pathways [31]. The reduced flavin adenine dinucleotide (FADH2) adds its electrons to the electron transport system at a lower level than NADH. During OXPHOS, electrons are transferred from the reducing equivalent NADH-FADH2 to O_2 in redox reactions via a chain of respiratory H^+ pumps. These pumps (complexes I-IV) establish an H^+ gradient across the inner mitochondrial membrane, and the energy of this gradient is then used to drive ATP synthesis by complex V [32]. During the electron transfer, $^{\cdot}O_2^-$ radicals are mainly generated at complexes I and III [33]. Mitochondria are endowed with a NOS (mtNOS) [34, 35] and thus $^{\cdot}NO$ and its derivatives are also produced by mitochondria where they have multiple effects that impact on cell physiology and death [36–38]. Mitochondrial ROS released into cytosol function as a second messenger to activate RIRR in neighboring mitochondria and potentially leading to apoptosis [8]. ROS are produced by various enzymatic pathways, mainly membrane-bound NADH and NADPH oxidases, xanthine oxidase, lipoxygenases, cyclooxygenases, and cytochromes P450 [39, 40]. ROS are also generated outside mitochondria during biotransformation of xenobiotics and drugs, inflammation, UV exposition, ionic irradiation, and lipid peroxidation of plasma membrane and other membrane–lipid structures [41].

1.4 Antioxidant Systems

Aerobic organisms are protected against ROS-induced oxidative damage by a network of highly complex and integrated endogenous enzymatic and non-enzymatic antioxidant systems. The key enzymes synthesized in the cell and directly involved in the control of ROS production are superoxide dismutases (SODs), catalase (CAT), glutathione peroxidases (GPXs), glutathione reductase (GSR), glucose-6-phosphate dehydrogenase (G6PD), and isocitrate dehydrogenases (IDHs). Glutathione, nicotinamide adenine dinucleotide phosphate ($NADP^+$), the reduced form of $NADP^+$ (NADPH), and sulfur-containing amino acids are the major non-enzymatic antioxidant systems that play key roles in protecting cells from oxidative stress. Aerobes are also protected from oxidative damage by various dietary antioxidants which are concentrated in foods of plant origin, mainly fruits and vegetables.

1.4.1 Endogenous Enzymatic Antioxidant Systems

The dismutation of $^{\cdot}O_2^-$, generated in different cellular compartments, into H_2O_2 and O_2 is the first step that plays a vital role in the control of cellular $^{\cdot}O_2^-$ production. Three distinct isoforms of SOD have been identified and characterized in mammals. Copper-zinc containing SOD (Cu, Zn-SOD or SOD1), a dimeric protein, was the first SOD discovered and characterized in eukaryotes cytoplasm [19]. Manganese containing SOD (Mn-SOD or SOD2) is located in the mitochondrial matrix [42]. The extracellular superoxide dismutase (EC-SOD) is a Cu- and Zn-containing tetrameric glycoprotein which is the major SOD in extracellular fluids such as plasma and lymph [43].

The control of H_2O_2 production is the second step that plays a vital role against ROS propagation. To prevent production and propagation of $^{\cdot}OH$ and $ONOO^-$, H_2O_2 generated after SOD catalyzing reaction is quickly converted into H_2O and O_2 by GPXs which have selenocysteine within its active site, and therefore they are selenium dependent for antioxidant activity. GPXs detoxify H_2O_2 to H_2O through the oxidation of Glutathione [44]. In addition, GPXs catalyze the degradation of lipid peroxides (LPO) and can metabolize lipid hydroperoxides to less reactive hydroxy fatty acids [44]. GPXs are therefore the primary antioxidant enzymes that protect biomembranes and cellular components against oxidative damage [45]. Glutathione exists in the reduced (GSH) and disulfide-oxidized (GSSG) states. GSR catalyzes the reduction of GSSG to GSH with NADPH as the reducing agent [44]; and it is therefore essential for the glutathione redox cycle that maintains adequate levels of GSH for GPX catalytic activity. GPXs present in the cytoplasm and the mitochondria, and CAT which found primarily within peroxisomes, both catalyze the conversion of H_2O_2 to H_2O.

NADPH required for the regeneration of GSH [46] is crucial for scavenging mitochondrial ROS through GSR and peroxidase systems. G6PD-produced NADPH pathway [47] is therefore crucial in the defense mechanism against oxidative stress. As a source of NADPH, mitochondrial and cytosolic NADP-IDHs play an important role in cellular defense against oxidative damage [48, 49]. The protective role of mitochondrial NADP+ dependent IDH against ROS-induced oxidative damage may be attributed to increased levels of a NADPH, needed for regeneration of GSH in the mitochondria [48]. The cytoplasmic NADP-linked IDH generates NADPH via oxidative decarboxylation of isocitrate, and thus potentially involved in the maintenance of the cellular redox state and plays an important role in the protection of cytoplasm components against ROS-induced oxidative stress [49].

The antioxidant enzymes and the small molecular weight non-enzymatic antioxidants represent coordinately operating cellular systems controlling ROS production [10] and prevent their propagation within and out of the cell (see Fig. 1.1).

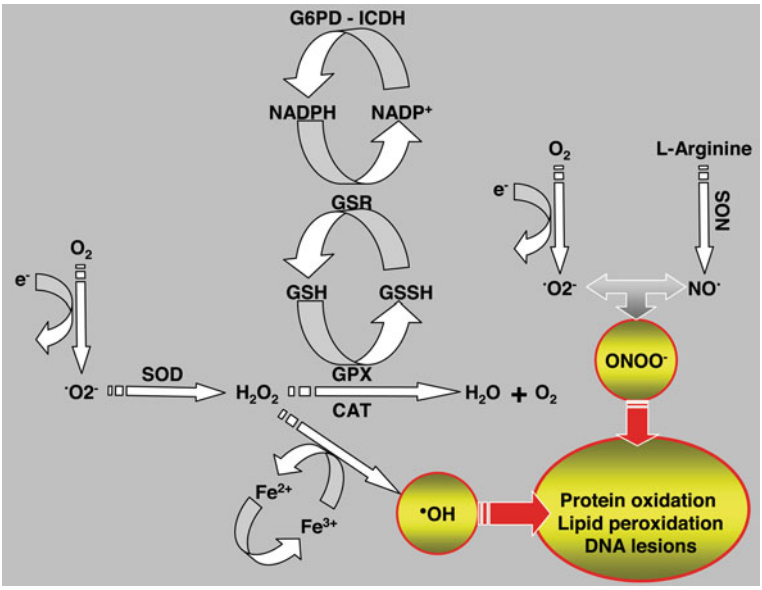

Fig. 1.1 Schematic representation of cellular reactive oxygen species (ROS) production and their control by enzymatic antioxidant systems. The production of superoxide anion ($^{\cdot}O_2^-$) by a single electron (e) donation to molecular oxygen (O_2) is the initial step in the formation and propagation of other ROS within and out of the cell. The major enzymes, namely copper-zinc containing superoxide dismutase (Cu, Zn-SOD or SOD_1), manganese containing SOD (MnSOD or SOD_2), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GSR), glucose-6-phosphate dehydrogenase (G6PD), and isocitrate dehydrogenases (ICDH) represent coordinately operating network of defenses against ROS-induced oxidative stress and cell damage. Hydrogen Peroxide (H_2O_2); water (H_2O); hydroxyl radical ($^{\cdot}OH$); peroxynitrite ($ONOO^-$); nitric oxide synthase (NOS); nitric oxide ($NO^{\cdot}$); reduced/oxidized glutathione (GSH/GSSG); reduced/oxidized nicotinamide adenine dinucleotide phosphate (NADPH/NADP)

1.4.2 Endogenous Non-Enzymatic Antioxidant Systems

The non-enzymatic antioxidants, NADH, NADPH, and GSH (as indicated above), and sulfur-containing amino acids are the key elements in the system of enzymatic antioxidant defenses against ROS-induced oxidative stress [50–54]. GSH has important roles as an independent free radical scavenger and antioxidant enzyme cofactor, as well as a cysteine carrier–storage form. Beside their role in protein synthesis, the sulfur-containing amino acids methionine and cysteine are also precursors of the key small molecular weight antioxidants, GSH and taurine. GSH is a tripeptide comprises glutamate, cysteine, and glycine. Cysteine is the rate-limiting substrate of GSH synthesis. Cysteine is a thiol-containing amino acid, which is also called a semi-essential amino acid because humans can synthesize it from an essential amino acid, methionine [55, 56]. Cysteine can also be obtained from the diet, usually as cystine which is a dimer of cysteine. Taurine is

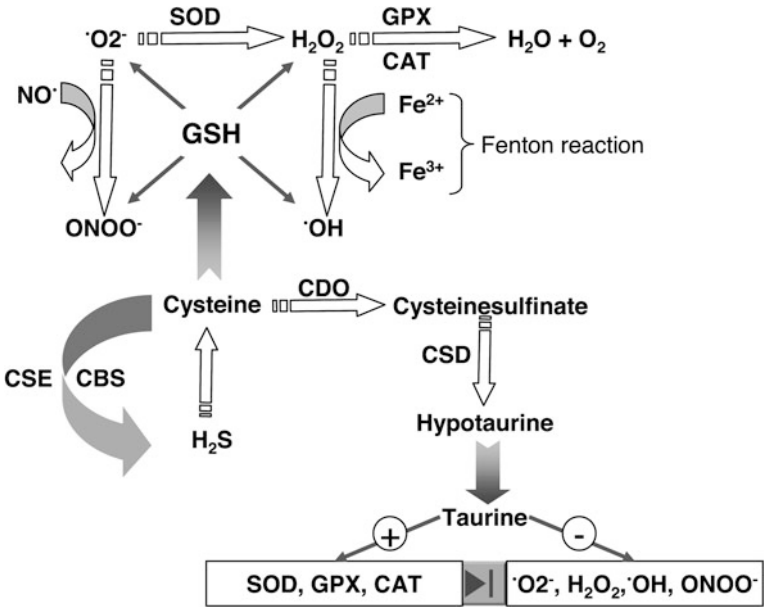

Fig. 1.2 Cytoprotective role of hydrogen sulfide (H_2S) against reactive oxygen species (ROS)-induced oxidative damage. H_2S is formed from cysteine by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). Then H_2S protects cells by two mechanisms: it enhances the production of the reduced form of glutathione (GSH) by enhancing cystine/cysteine transport and redistributes them to the mitochondria. Molecular oxygen (O_2); superoxide anion ($^{\cdot}O_2^{-}$); hydrogen peroxide (H_2O_2); water (H_2O); hydroxyl radical ($^{\cdot}OH$); peroxynitrite ($ONOO^{-}$); nitric oxide ($NO^{\cdot}$); superoxide dismutase (SOD); catalase (CAT); glutathione peroxidase (GPX), cysteine dioxygenase (CDO), cysteinesulnate decarboxylase (CSD)

synthesized from cysteine via the actions of cysteine dioxygenase (CDO), which gives rise to cysteinesulnate, and cysteinesulnate decarboxylase (CSD), which decarboxylates cysteinesulnate to hypotaurine [57, 58]. Hypotaurine is further oxidized to taurine (see Fig. 1.2). Taurine prevents oxidant-induced cell damage by direct upregulation of key antioxidant enzymes and attenuation of ROS generation [52, 53], and plays an important cytoprotective role [59].

Hydrogen sulfide (H_2S), an endogenous antioxidant gas [60], is recognized as an important signaling molecule [61, 62] with therapeutic potential [63, 64]. H_2S has been shown to be produced by various mammalian organs [65], including the reproductive tissues [66]. It is formed from cysteine by pyridoxal-5′-phosphate (PLP)-dependent enzymes, namely cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) [67]. Importantly, H_2S protects cells against oxidative stress [68] by two mechanisms: it increases the production of GSH by enhancing cystine/cysteine transport and redistributes them to the mitochondria [69]. The cytoprotective role of H_2S [70–72] is therefore crucial for preventing ROS accumulation and oxidative damage (see Fig. 1.2).

1.4.3 Exogenous Antioxidant Systems

Nutrient antioxidants are diverse compounds characterized by their ability to quench ROS. Common dietary antioxidants include vitamins, trace elements, and polyphenols. Dietary antioxidants are classified into two types, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). Water-soluble antioxidants are present in the cytoplasm and the blood plasma, while lipid-soluble antioxidants are mainly present in cell membrane and cellular organelles where they protect cells from lipid peroxidation.

Carotenoids (vitamin A), Ascorbate (vitamin C), and tocopherols (vitamin E) are among the major dietary antioxidants that scavenge directly extracellular ROS and provide a major source of protection against their damaging effects [73–78]. Carotenoids, such as ß-carotene, lutein, α-carotene, zeaxanthin, cryptoxanthin, and lycopene, are fat-soluble antioxidants [73, 74]. ß-carotene is the main source of provitamin A. Green leafy vegetables, carrots, and other yellow root vegetables, and yellow and orange fruits contain ß-carotene. ß-carotene, and lycopene are important biological compounds that can inactivate electronically excited molecules, a process termed quenching, and may also participate in free radical reactions [79]. Vitamin C is a water-soluble antioxidant which is present in various fruits and vegetables. Ascorbic acid has been shown to interact with the tocopheroxyl radical and to regenerate the reduced tocopherol [80]. α, β, γ, and δ-tocopherol and the corresponding four tocotrienols are naturally occurring fat-soluble dietary vitamins with antioxidant properties [81]. The natural sources of vitamins E are vegetable oils. The most biologically active form of the vitamin E homologs is α-tocopherol. It contributes in the protection of cell membrane polyunsaturated fatty acids (PUFA) against ROS-induced peroxidative damage [82], and thus plays important role in membrane stability and functions [83–85]. It is important to highlight that vitamins C and E interact synergistically in association with GSH-related enzymes to control the production of lipid peroxidation products (see Fig. 1.3).

Trace elements are present in minute quantities in the body and are required in low concentrations in the diet [86]. Some elements form part of the active site necessary for the antioxidant enzyme function, act as cofactors in the regulation of antioxidant enzymes, or shaping the enzyme configuration necessary for its activity. Among trace elements, Cu, Zn, Mn, and Se are essential micronutrients with wide range of functions in the body including the synthesis and activity of antioxidant enzymes, namely Cu, Zn-SOD (cytosolic SOD1), Mn-SOD (mitochondrial SOD2), and four Se-GPX isoforms (cytosolic GPX1, gastrointestinal GPX or GPX2, plasma GPX or GPX3, phospholipid hydroperoxide GPX or GPX4).

Polyphenols are the most abundant antioxidants in the human diet. They are a wide variety of organic molecules that naturally occurring in vegetables, fruits, and plant-derived beverages such as tea, red wine, and olive oil [87]. Polyphenols are characterized by the presence of several groups involved in phenolic structures

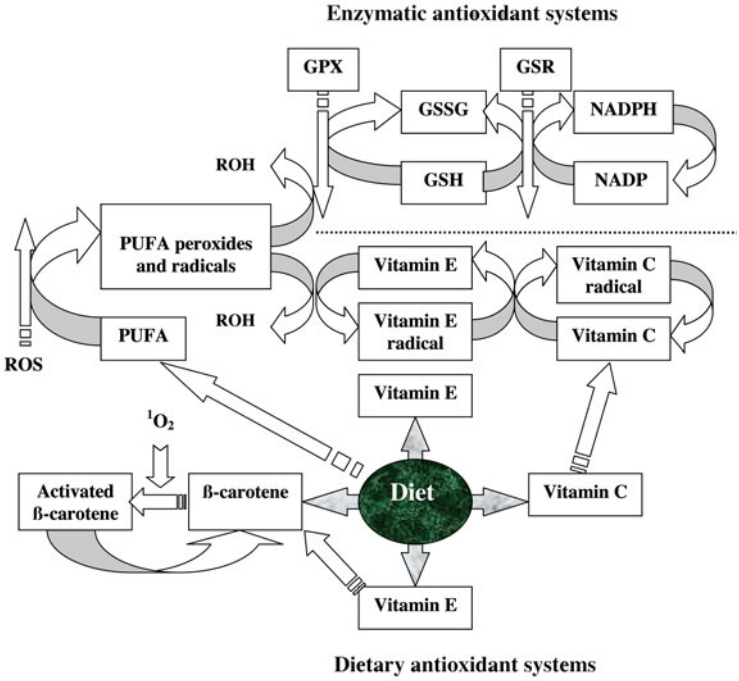

Fig. 1.3 The reaction sequences between dietary antioxidant vitamins and enzymatic antioxidants ensuring cellular defense against reactive oxygen species (ROS) and lipid peroxide. Glutathione peroxidase (GPX); glutathione reductase (GSR); reduced/oxidized glutathione (GSH/GSSG); reduced/oxidized nicotinamide adenine dinucleotide phosphate (NADPH/NADP), polyunsaturated fatty acids (PUFA); singlet oxygen ($^1O_2$); alcohol (ROH). Adapted from Machlin and Bendich [80]

and include the flavonoids and phenolic acids such as catechins, resveratrol, quercetin, anthocyanins, hesperitin derivatives, phytic, caffeic, and chlorogenic acids. Pigmented fruits, such as pomegranates, grapes, apples, berries, pears, cantaloupe melon, and watermelon, and vegetables, such as broccoli, cabbage, celery, onion, and parsley, are rich in polyphenol antioxidants. Phytic acid, by virtue of chelating free iron, is a potent inhibitor of $^{\cdot}OH$ formation by the Fenton reaction and accelerates O_2-mediated Fe^{2+} depletion and suppress iron-mediated oxidative processes. Various polyphenolic compounds with antioxidant activity are potential contributors to explain the human health benefits of diets rich in fruits and vegetables. Dietary polyphenols, in addition to their direct ROS scavenging action, can protect cells against ROS oxidative damage by increasing the expression of mRNA encoding the key antioxidant enzymes [88].

1.5 ROS and Antioxidants Cross-Talk in Prenatal Developmental Outcomes

The establishment of pregnancy in mammals can be divided into four developmental periods: (1) fertilization of the oocyte and early embryonic development, (2) implantation of the developing blastocyst, (3) post-implantation embryonic development, and (4) Fetal and placental development. Mitochondrial OXPHOS is a vital metabolic pathway that uses energy released by the oxidation of nutrients to produce ATP to meet high energy demand of the conceptus (embryo/fetus and associated placental membranes) during its metabolic and developmental processes [89, 90]. During early embryonic development, mitochondria also provide ATP for the regeneration of NADPH and GSH [91].

Before implantation, embryonic development and growth occurs under uterine hypoxic environment and embryonic tissues cannot produce large amount of ATP. The switch from preimplantation embryonic anaerobic metabolism to post-implantation aerobic metabolism induced by conceptus vascularization onset and uteroplacental blood flow exposed embryonic and extraembryonic tissues to ROS produced as normal by-products of OXPHOS. These changes take place during the period of organogenesis, when the conceptus is potentially vulnerable to environmental factors. Post-implantation conceptus may be therefore particularly vulnerable to oxidative stress early in pregnancy. The control of ROS production is important in development through cellular signaling pathways involved in proliferation, differentiation or apoptosis, whereas high ROS levels induces oxidative stress and can alter embryonic development [92]. Mitochondrial dysfunction due to pathologic and/or environmental insults triggers apoptosis in the embryo and compromises early developmental processes and birth outcome [91, 93]. Abnormal or reduced mitochondrial activity alters ROS production and reduces implantation rates in women [94]. Therefore, antioxidant status of the conceptus and its surrounding play vital roles in protecting embryonic and extraembryonic tissues from the deleterious effects of endogenous ROS and those generated after in utero exposure to environmental factors during the critical period of early prenatal development.

Embryonic implantation is promoted by a network of signaling molecules that mediate cell-to-cell communications between the receptive endometrium and embryonic trophectoderm. Animal studies, mainly in rodents, indicate that ROS production from both mitochondrial and non-mitochondrial sources, functions as a physiologic component of the early embryonic development. GSH-dependent antioxidant mechanisms are developmentally regulated in the inner blastocyst cell mass and H_2O_2 is a potential mediator of apoptosis in the blastocyst [95]. Pre- and post-implantation mouse embryos generate and release ROS [96, 97]. An abrupt drop in SOD activity and a concomitant rise in $^{\cdot}O_2^-$ levels occur in mouse blastocyst at the perihatching stage [98]. The uterine $^{\cdot}O_2^-$ burst at proestrus in rat suggested its involvement in regulating uterine edema and cell proliferation [99]. A peak of $^{\cdot}O_2^-$ in the uterus at day five of mice pregnancy suggested a contribution of this radical in vascular permeability at the initiation of implantation [100]. NADPH-dependent $^{\cdot}O_2^-$ production pathway associated with the uterus of pregnant mice increases across the

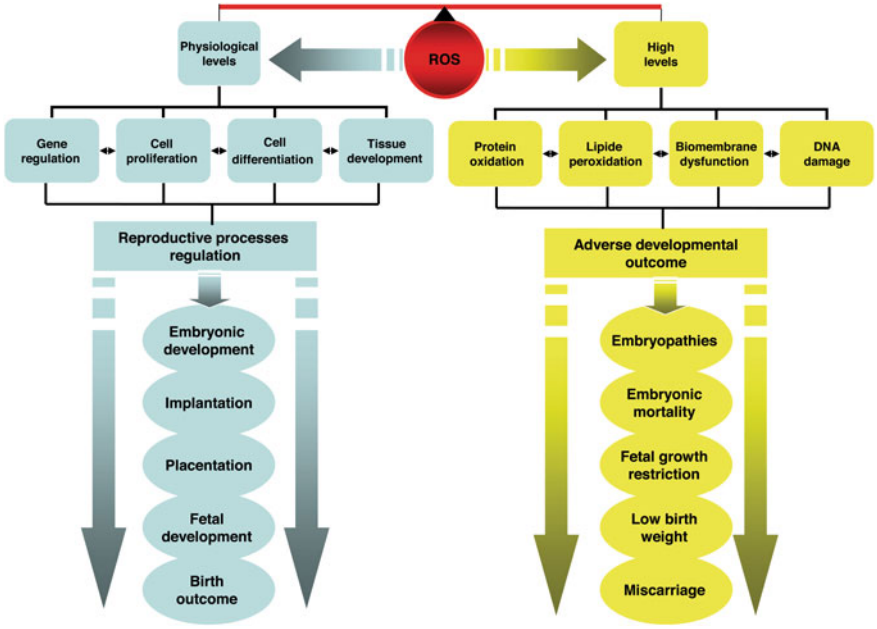

Fig. 1.4 Schematic overview of physiologic roles of reactive oxygen species (ROS) in normal reproductive processes or induced adverse developmental outcomes. Controlled physiologic level of ROS plays important roles in developmental processes and birth outcome through the regulation of gene expression, cell proliferation and differentiation, and tissue development. High levels of ROS damage cellular macromolecules and membranes which lead to adverse developmental outcomes, disorders, and miscarriage

preimplantation stages [101]. Antioxidant enzyme systems have been shown as components of the developing embryo and its receptive uterine endometrium [102–107] and may have vital role in regulating fetal development and survival through the control of placental ROS production and propagation [108–112].

Physiologic levels of ROS play important role in the regulation of reproductive processes, including embryonic development, uterine receptivity, embryonic implantation, placental development and endocrine functions, and fetal development, whereas high ROS levels have adverse developmental outcome, including embryopathies, embryonic mortality, fetal growth restriction, low birth weight, and miscarriage (see Fig. 1.4).

1.6 Maternal Dietary Antioxidants and Prenatal Developmental Outcomes

Maternal dietary antioxidants are essential nutrients for embryonic and fetal development and health outcomes. Adequate maternal antioxidant status before and during pregnancy could prevent oxidative mechanisms induced by poor

dietary nutritional factors, importantly micronutrient antioxidants. Maternal under- or poor nutrition are associated with multiple micronutrient deficiencies, a major cause of IUGR and miscarriage [113]. Maternal nutritional factors play a major role in programing the susceptibility of offspring to oxidative stress and disease [16]. Maternal diet composition is important determinant of the balance between antioxidants and ROS that affect prenatal growth and development, [114, 115]. Maternal antioxidant status has a positive influence on birth weight [116].

Under conditions of low dietary intake, maternal nutritional deficiencies, or suboptimal maternal nutritional status, trace elements and/or antioxidant vitamins during pregnancy can be compromised [117–119]. Deficiencies and or alterations in trace elements and antioxidant vitamins, associated with oxidant/antioxidant imbalance, impair fetoplacental development, and have persistent effects on fetal development and neonatal tissues [120–124]. Trace elements deficiency influences prenatal development through several mechanisms including ROS-induced oxidative damage that is secondary to compromised antioxidant defense systems. The activities of Cu, Zn-SOD (SOD1), Mn-SOD (SOD2) and Se-GPX are sensitive to dietary deficiency in Cu, Zn, Mn, or Se. Low SOD activity in Cu-deficient rat embryos exposes the embryo to excessive ROS production, resulting in subsequent cellular oxidative stress, a shift in the redox state to a more oxidized environment, embryonic damage, and developmental defects [125]. Maternal Cu deficiency is associated with multiple fetal developmental defects that can affect the central nervous system, cardiovascular, and skeletal systems, and result in poor immunocompetence and behavioral abnormalities of the offspring [126]. Cu deficiency induce decreased SOD activity and increased $\cdot O_2^-$ and $ONOO^-$ concentrations that can lead to nitration of proteins and alterations in protein function and activity [127]. A decrease in NO bioavailability can inhibit NO-mediated intracellular signaling [128] and adversely affect embryonic and fetal development [129]. Deficiencies of Zn, Cu, and Mn have been implicated in human reproductive disorders like infertility, pregnancy wastage, pregnancy induced hypertension, premature rupture of extraembryonic membranes, and low birth weight [130]. Malnutrition is associated with oxidative stress in small for gestational age neonates born at term to malnourished mothers [131]. Poor maternal dietary antioxidants have adverse prenatal developmental outcomes and play a role in programing the susceptibility of offspring to disease (see Fig. 1.5).

Antioxidant vitamins and minerals interventions during prenatal development are important for preventing the damage caused by ROS in utero [132]. Animal studies have shown that one selected antioxidant or combined with other supplements decreases embryonic mortality and improves birth outcomes [133–135]. Blood α-tocopherol concentrations are lower in abnormal pregnancies than those in normal ones, suggesting that vitamin E requirements increase throughout pregnancy [136]. Nevertheless, the association between dietary antioxidants and birth outcomes is not conclusive in human or animal studies [137–139]. Prophylactic use of some micronutrients may be useful in preventing adverse pregnancy outcomes [140]. Nevertheless, early human pregnancy supplementation with pharmacologic doses of antioxidant vitamin E is associated with a decrease in birth

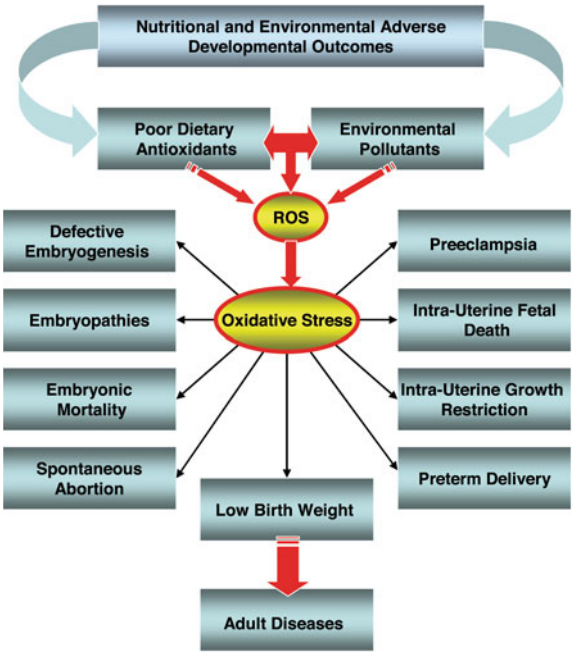

Fig. 1.5 Reactive oxygen species (ROS)-induced oxidative stress caused by poor dietary antioxidant intakes and/or environmental pollutants in foods, and the subsequent adverse developmental outcomes and pregnancy-associated disorders and complications

weight [141]. Maternal supplementation with vitamins C and/or E does not prevent preeclampsia in high-risk pregnant women [142, 143]. Furthermore, high maternal consumption of selected dietary antioxidants does not protect the child from development of advanced β cell autoimmunity in early childhood [144]. The reason for the failure of such supplementations to improve the prenatal developmental outcomes, and maternal and child health could be imbalanced administration of antioxidant vitamins and/or trace elements. The benefits of using dietary supplement that contains one or a limited number of antioxidants on prenatal developmental outcomes remain far from efficient and inconclusive. Consumption of various antioxidants in foods is likely more effective than limited number of antioxidants or large doses of a single antioxidant given for a finite period.

The diet is an important source of diverse antioxidants. Antioxidant-rich foods, specifically high consumption of fruits, vegetables, cereals, and plant-derived oils can prevent and delay the development of chronic age-related diseases [145]. An interesting example worth mentioning is the traditional Mediterranean diet which is rich in antioxidants components, mainly vitamins, essential trace elements, and polyphenols [146]. Mediterranean diet is widely reported as a nutritional model that keeps overall maintenance of human health and protection from disease [147–149] through the lowering of prooxidant-induced oxidative stress [150–152]. This diet is essentially composed of high intake of legumes, fruits, vegetables, cereals, and nuts, high to moderate consumption of fish, moderate consumption of dairy products and wine, and low consumption of meat and meat products.

Mediterranean diet seems to play an important role in preventing chronic diseases [153, 154]. Mediterranean-type diet can be proposed as a mean to improve fertility [155] and decrease risk of premature labor and gestational diabetes [156]. Despite the fact that plant roots, stems, leaves, flowers, fruits, and seeds consumption can improves dietary quality in women before conception and during the first trimester of pregnancy, little is known about how these wide variety of antioxidant-rich edible dietary sources affects favorably prenatal developmental outcomes.

1.7 Environmental Contaminants and Prenatal Developmental Outcomes

Industrial and agricultural activities contribute to the release of large quantities of various chemical in the environment and have resulted in widespread air, soil, and water contamination. Exposure to such environmental contaminants is inevitable as it occurs through the consumption of contaminated food, water, beverages. There is substantial evidence that many human chronic diseases results from exposure to environmental factors early in development [157]. Environmental chemicals disrupt endocrine function and contribute to alterations in growth and development [158, 159]. In utero exposure to environmental chemicals can mediate adverse prenatal developmental and birth defect [160]. Various environmental chemicals, to which the mother is exposed during the critical periods of pregnancy [17], pass across the placental barrier into the fetal blood stream and can be transferred to the fetus [18]. The fetus is particularly vulnerable to the adverse effects of environmental chemicals [17]. Embryonic and fetal period are vulnerable to oxidative stress and many environmental pollutants may contribute to adverse prenatal developmental outcomes and can lead to chronic health problems later in life (see Fig. 1.5), at least in part, via ROS generation which damage cellular macromolecules and/or alter signal transduction pathways [18, 161]. Environmental chemical exposure during development induces oxidative stress and fetal toxicity that adversely affects fetal ovarian development [162], contribute to birth defects [163] and may ultimately lead to cancer later on in life [164].

The developing organism is susceptible to oxidative stress induced by chemical contaminants due to its inadequate antioxidant defense systems [165]. The toxicity, dosage, and timing of exposure to environmental chemicals are important determinants of adverse in utero effects on the developing organs and tissues. ROS-induced oxidative stress has emerged as a promoter of prenatal developmental disorders, such as embryopathies, embryonic mortality, abortion, fetal growth restriction, and low birth weight [9, 10]. Chemicals in food may contribute to such adverse prenatal outcomes and increase the susceptibility of offspring to disease [164] via impairment of the antioxidant defense systems and enhancement of ROS generation [16]. However, the relationship between oxidative stress induced by environmental chemicals and the adverse prenatal developmental

outcomes is not clear and cannot be investigated in human pregnancies for evident ethical reasons. Animals, mainly rodents, have been often used to examine the adverse developmental outcomes of chemical contaminants.

The man-made chemicals, importantly the estrogen-mimic bisphenol-A (BPA) to which the mother is exposed during the critical periods of pregnancy, pass across the placenta and can be transferred to the fetus [166–168]. BPA is the main monomeric chemical used in the production of polycarbonate plastic, the manufacture of food-storage containers and the epoxy resin that form the lining of food and beverage cans and dental sealants. This endocrine disruptor [169] can be leached from plastic, food cans, and containers and widely spread in the environment and food chain. Of particular concern is the contamination by BPA during pregnancy that is evidenced by its presence in maternal and umbilical cord blood, amniotic fluid, fetal and placental tissues [170, 171]. The mouse brain, kidney, liver, and testes display high levels of H_2O_2 following BPA exposure [172]. In these organs, BPA-induced oxidative stress occurs, at least in part, through distribution of the redox control systems, mainly the levels of GSH/GSSG [172]. BPA exposure during embryonic/fetal development in rodents induced oxidative stress and ultimately leads to underdevelopment of fetal brain, kidney, and testis [173] and disturbs postnatal reproductive functions [174, 175]. Prenatal BPA exposure of ewes, at levels similar to that seen in human maternal circulation resulted in low birth weight [176]. All these perturbations raise the question of the impact of maternal exposure to BPA on the development process, the risk of fetal growth restriction and consequently the risk of developing endocrine and reproductive disorders throughout adult life.

The actions of estrogen and progesterone via their uterine receptors orchestrate the changes in the mammalian endometrium that make it receptive to the blastocyst implantation and the establishment of pregnancy. Exposure of mice to BPA early in pregnancy acts at the uterus to disrupt intrauterine implantation, consistent with an estrogenic BPA action [177]. Estrogen biosynthesis is catalyzed by aromatase cytochrome P450, the product of the CYP19 gene. BPA is an estrogen receptor antagonist and inhibit estrogen synthesis by downregulation of CYP19. The BPA-induced changes in the expression of sex steroid receptors have consequences for the hormonal responsiveness of the mice uterus to both endogenous and exogenous hormones, and the potential for predisposition of the organ to disease later in life [175]. The activities of antioxidant enzymes in the sheep endometrium are regulated by estradiol [107]. BPA has been shown to downregulate CYP19 expression in placental cells [178]. The early luteo-placental shift in progesterone production plays crucial role in maintenance of pregnancy beyond the corpus luteum life span in humans [179] and sheeps [180], allowing continued prenatal development. Increased placentome progesterone content early in pregnancy [111] indicates that the steroidogenic capacity of placentome cells increased as pregnancy advances. ROS inhibit steroidogenesis by blocking cholesterol transport into mitochondria [181]. The increases in antioxidant enzyme activities and progesterone production in ovine placentomes during early pregnancy [111] indicates that antioxidant systems act as a protective mechanism against ROS-