Review

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Oxidative Stress Factors is Effective on Reproductive Functions: A Review

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ABSTRACT

Oxidative stress (OS) results from an imbalance in the production of reactive oxygen species (ROS) and antioxidant defenses, leading to cellular damage, caused by excess ROS. OS is mediated by free radicals, such as ROS or reactive nitrogen species produced during physiological aerobic metabolism and pathological inflammatory processes. OS causes damage to cellular components, inactivation of essential metabolic enzymes and disruption of signal transduction pathways.

OS has been shown to play an important role in the pathogenesis of subfertility in both men and women. OS has been associated with a number of reproductive diseases such as endometriosis, polycystic ovary syndrome and unexplained infertility. Pregnancy complications, such as spontaneous abortion, recurrent pregnancy loss and pre-eclampsia may also develop in response to OS. Antioxidant supplementation may be effective in controlling ROS production and continues to be explored as an option to overcome reproductive disorders associated with infertility.

Keywords: Antioxidants, female infertility, oxidative stress, reactive oxygen species

INTRODUCTION

Oxygen (O_2) is essential for aerobic life and oxidative metabolism is the main source of energy. Cells have a defense system against reactive oxygen species (ROS) under aerobic conditions and there is a homeostasis between pro-oxidants and antioxidants for healthy biology. Oxidative stress (OS) occurs with excessive ROS production or when the anti-oxidant defense mechanisms are weakened.[1,2] The most biologically important ROS are superoxide anion (O_2--), hydroxyl radical (-OH), peroxyl, alkoxyl and hydroperoxyl. Free radical species are unstable and highly reactive, but can become stable by taking electrons from lipids, nucleic acids, proteins, carbohydrates or other nearby molecules, causing chain reactions and leading to cellular damage and disease.[3] Therefore, OS can cause DNA damage, lipid peroxidation and also protein damage. Under normal conditions, there are two types of antioxidants in the body; non-enzymatic antioxidants and enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase (GSR), which can cause the reduction of hydrogen peroxide (H_2O_2) to water and alcohol. Non-enzymatic antioxidants are synthetic antioxidants or dietary supplements containing vitamin C, vitamin E, β-carotene, selenium, Zn, taurine, glutathione (GSH) and the like.[4]

OS is thought to be responsible for the initiation or development of pathological processes[5] affecting female reproduction, such as embryonic resorption, recurrent pregnancy loss, preeclampsia, intrauterine growth restriction and fetal death.[6] A non-pathological level of ROS is an important regulator of folliculogenesis, corpus luteum oocyte maturation and feto-placental development through various signaling pathways.[7] However, ROS can sometimes exert deleterious effects when present in excessive amounts. Thus, ROS have a close relationship with reproductive events. Excessive ROS may lead to multiple reproductive problems, including endometriosis, polycystic ovary syndrome (PCOS) and unexplained infertility.[8] Is also a central element of cell signaling, gene expression and signal transduction pathways involved in cell function, growth, differentiation and death.[9] Therefore, tightly controlled ROS

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production is important for normal function and good health.

Reactive Oxygen Species and Their Physiological Actions

ROS are produced during O_2 consumption.[10] ROS are composed of free and/or non-free radical intermediates, with free radicals being the most reactive. This reactivity is due to one or more unpaired electrons in the outer shell of the atom. In addition, O_2- and nitrogen-dependent biological processes are of greater importance because their end products are often found in pathological processes or in situations of high metabolic demand in external environmental interactions.[1]

Biological systems contain large amounts of O_2. As a radical, O_2 reacts rapidly with other radicals. Free radicals are usually produced from O_2 itself and partially reduced species originate from normal metabolic processes in the body. ROS are prominent and potentially toxic intermediates commonly found in OS.[11]

The Haber-Weiss reaction, given below, is the main mechanism by which the highly reactive hydroxyl radical (OH*) is produced. This reaction can generate more toxic radicals through interactions between the superoxide (SO) anion and H_2O_2. There are three main types of ROS: superoxide anion (O_2--),H_2O_2 and hydroxyl (-OH).[11,12]

$O+-2+H_2O_2>O_2+OH-+OH^*$

However, this reaction has been found to be thermodynamically inefficient in biological systems.

The Fenton reaction, which consists of two reactions, demonstrates the use of a metal ion catalyst to produce OH* as shown below.[11]

$Fe_3++O.-2>Fe_2++O_2$

$Fe_2++H_2O_2>Fe_3++OH-+OH^*$

Most ROS are produced when electrons leak from the mitochondrial respiratory chain, also called the electron transport chain (ETC).[10] According to one study, up to 2% of the O_2 consumed by mitochondria can be diverted to ROS formation, especially in complexes I and III.[13] The free radical superoxide anion (O_2--) is formed when an electron is added to ground state dioxygen, but is unstable in aqueous solutions because it can react spontaneously to produce H_2O_2 and molecular O_2. It can reduce Fe_3+ to Fe_2+ and convert to O_2. H_2O_2 is not a free radical, but it is highly damaging to cells as it can cross biological membranes and convert to the highly reactive -OH.[14]

Physiological processes that utilize O_2 as a substrate, such as oxygenase reactions and electron transfer reactions, produce large amounts of ROS, the most common of which is the SO anion.[15]

Other sources of SO anion include the short electron chain in the endoplasmic reticulum, cytochrome P450 and the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and other oxido-reductases that are produced in excess, especially in early pregnancy.[1,10]

Mitochondria are of central importance for metabolic activities in cells, so a disruption in their function can cause significant alteration in adenine triphosphate (ATP) production. The energy derived from ATP is essential for gamete function. Although mitochondria are the main sites of ROS production, excess ROS can alter the function of mitochondria in oocytes and embryos. This mitochondrial dysfunction can cause cell division arrest triggered by OS.[16] A slight increase in ROS levels stimulates cell growth and proliferation and allows normal physiological processes. Conversely, excess ROS causes cellular damage (e.g., damage to DNA, lipid membranes and proteins).

Reactive Nitrogen Species

Reactive nitrogen species (RNS) include nitric oxide (NO) and nitrogen dioxide in addition to non-reactive species, such as peroxynitrite (ONOO-) and nitrosamines. Peroxynitrite may induce lipid peroxidation and nitrozation of many tyrosine molecules that normally act as mediators of enzyme function and signal transduction.[17]

NO is a free radical with vasodilatory properties. Although the vasodilatory effects of NO can be therapeutic, excessive RNS production can affect protein structure and function. NO-related effects have also been suggested to occur through ROS production through the interaction between NO and the SO anion. In the absence of L-arginine[17] and in sustained low antioxidant states, intracellular production of SO is increased. Elevated SO anion levels promote reactions between peroxynitrite formation and NO, which exacerbates cytotoxicity.[18]

The enzyme nitric oxide synthase (NOS) uses NADPH as an electron donor, catalyzes NO formation from O_2 and L-arginine[19] and is composed of the following isoforms: neuronal NOS (nNOS or NOS I), inducible NOS (iNOS or NOS II) and endothelial NOS (eNOS or NOS III). The nNOS isoform is a neurotransmitter and iNOS is expressed primarily in macrophages following induction by cytokines. eNOS activity increases in response to luteinizing hormone (LH) surge and human chorionic gonadotropin.[10] In general, NO produced by eNOS and nNOS regulate physiological functions, whereas NO production by iNOS appears to be more active in pathophysiological conditions.

Antioxidant Defense Mechanisms

The enzymatic antioxidants SOD, GPx, CAT and GSR all function as endogenous elements of the primary antioxidant defense mechanism. SOD catalyzes O_2-- dismutation to produce H_2O_2 and O_2 at a rate 104-fold higher than normal dismutation at physiological pH.[20] CAT serves to remove H_2O_2 from the cell when H_2O_2 is in high concentrations. GPx catalyzes the reduction of H_2O_2 and organic free hydroperoxides but requires GSH as a co-substrate. GSR is a cytosolic protein with a tissue distribution similar to that of GPx. GSR also reduces oxidized GSH using NADPH produced by various systems.[7,21]

Non-Enzymatic Antioxidants

Non-enzymatic antioxidants consist of metabolites absorbed from the diet or synthetic antioxidant compounds, including vitamin C, GSH, taurine, hypotaurine, vitamin E, zinc (Zn), selenium, beta-carotene and carotene.[22]

Vitamin C (ascorbic acid) is a redox catalyst that is involved in the neutralization of ROS by reduction. Its reduced form is protected by reactions with GSH and can also be catalyzed by the enzyme disulfide isomerase and glutaredoxins.

GSH is a peptide made in the cytosol from cysteine, glutamate and glycine and is present in most forms of aerobic life.[23] Of note, it is also the main non-enzymatic antioxidant found in oocytes and embryos. The source of its antioxidant property is the thiol group of its cysteine component, which is a reducing agent that allows it to be reversibly oxidized and reduced to its stable form.[24] GSH levels are regulated by *de novo* formation catalyzed by the enzymes gamma-GCS and GSH synthetase.[2] GSH participates in multiple reactions, including the formation of GSH disulfide, which is converted back to GSH by GSR at the expense of NADPH.[21]

Cysteine and cysteamine (CSH) increase the GSH content of the oocyte. CSH also acts as a scavenger and is necessary to maintain high GSH levels. CSH can also be converted to hypotaurine, another antioxidant. The concentrations of many amino acids, including taurine, vary significantly during folliculogenesis. Taurine and hypotaurine are scavengers that help maintain redox homeostasis in the gametes.[24]

Vitamin E, the major lipid-soluble vitamin found in all cell membranes, provides effective protection against lipid peroxidation. The tocopheryl radical can be directly reduced by a ascorbic acid-GSH redox couple. β-carotene shows the most effective scavenging effect, together with vitamin E, but β-carotene is more effective at low O_2 pressures. However, vitamin E prevents oxidation of the conjugated double bonds of β-carotene.[14]

Unlike vitamins C and E and GSH, the hormone melatonin is an antioxidant that can be produced by the human body. However, it cannot enter the redox cycle like other antioxidants; once oxidized, melatonin cannot return to its reduced state. It forms stable end products after the reaction. The iron-binding proteins transferrin and ferritin prevent the catalysis of free radicals through chelation and play an important role in antioxidant defense.[25] Although nutrients such as Se, copper (Cu) and Zn do not have antioxidant effects, they are necessary for the activity of some antioxidant enzymes.

Oxidative Stress and Antioxidant Capacity in the Male Reproductive System

Almost half of infertility cases may be due to male reproductive pathologies, which can be congenital or acquired.[26] In men, the role of OS has long been recognized as a major contributing factor to infertility. Men with high OS levels or DNA-damaged sperm are more likely to experience infertility. The key determinants of fertilization capacity are sperm count and motility. These key factors can both be impaired by ROS.[27]

There is much evidence to support the role of ROS in male infertility.[28,29] Spermatozoa have a unique plasma membrane structure that contains significant levels of polyunsaturated fatty acids that also increase membrane flexibility, which is essential for oocyte penetration. Unfortunately, this membrane is highly vulnerable to ROS attack.[29,30] The precise reaction appears to be a cascade of lipid peroxidation that compromises membrane cell integrity, causing a decrease in sperm motility and subsequent reduced fertility. In addition, ROS also lead to significant DNA damage.[31]

Natural antioxidants in the human body include vitamins C and E, SOD, thioredoxin and GSH. These antioxidants can neutralize free radical activity and protect spermatozoa from ROS.[32] There may be lower concentrations of antioxidants in the semen of infertile men. This may explain the high levels of ROS in the semen of infertile men compared to fertile men. Sperm function tests such as sperm DNA fragmentation and OS measurements are widely used to provide a better understanding of true male fertility potential.[33]

Semen analysis may show the following pathologies: asthenospermia (reduced motility), oligozoospermia (low sperm count) and teratozoospermia (abnormal morphology) or a combination of all of these (WHO 2021 classification). The WHO 2021 guidelines recommend the standard values of sperm parameters are: total sperm count 39 million or more per ejaculate; sperm concentration ≥16 million/mL; sperm volume 1.4 mL or more; progressive sperm motility 30% or more; and normal morphology 4% or more.[34]

DISCUSSION

Vitamin C acts as an important co-factor in hydroxylation and amidation reactions and is a water-soluble antioxidant.[35] Together with vitamin E, it plays an important role in collagen synthesis, proteoglycans and intercellular matrix.[36] Vitamin C is found in high concentrations in seminal fluid.[37]

Greco et al.[38] reported an intervention study with men suffering from infertility. In this study, the intervention group received one gram of vitamin E and one gram of vitamin C. After two months, there was a significant reduction in the degree of DNA damage in the intervention group ($p<0.001$). However, no positive correlation was found between vitamin E and C treatment and the key semen parameters, motility and concentration. In a second study by these authors, intracytoplasmic sperm injection and in vitro fertilization show that a significant proportion of sperm DNA damage leads to lower infertility rates using the same antioxidant supplementation as in the earlier study (vitamin E 1 g and vitamin C 1 g).[38] Moslemi and Tavanbakhsh[39] performed a study with 690 infertile men with idiopathic Oligoasthenoteratozoospermi (OAT) receiving daily antioxidant supplementation with selenium (200 μg) in addition to vitamin E (400 IU). Supplements were given for at least 100 days. The authors reported an overall improvement in sperm motility, morphology, or both in 52.6% (362 cases) and a spontaneous pregnancy rate of 10.8% (75 cases) compared to men receiving no treatment.

L-carnitine (LC) (3-aminobutyric acid) is present in the human body and is also a metabolized vitamin. The participation of LC in intermediary metabolism is essential and plays an important role in the formation of acyl carnitine esters.[40] High concentrations of LC in the human body are 2000 times higher in the epididymis than in serum.[41]

To date, few studies have investigated the effect of LC supplementation. Lenzi et al.[42] reported a double-blind controlled clinical trial on the effect of LC on male infertility. In

this study, 60 infertile men with OAT were treated, divided into intervention and control groups. Patients in the intervention group received 2 g/day LC and 1 g/day L-acetyl carnitine (LAC) for six months. A positive association between LC and LAC supplementation and sperm motility was observed in the study population. Interestingly, this association was more significant with lower sperm motility at baseline sperm quality assessment. Garolla et al.[43] reported the effect of LC treatment and phospholipid hydroperoxide glutathione peroxidase (PHGPX) treatment in men with OAT. In this study, 30 men with idiopathic OAT were treated in a double-blind study. They formed two patient groups. One group of patients was treated with placebo for three months followed by 2 g LC daily for another three months. Those receiving LC treatment showed improvement in sperm motility when there was normal PHGPX levels.

Co-enzyme Q10 (CoQ10, also known as ubiquinone) is another antioxidant supplement. As an ETC component, it is involved in aerobic cellular respiration producing cellular energy compounds such as ATP. Balercia et al.[44] reported the effect of CoQ10 on sperm motility in infertile men. In their study, 60 men with idiopathic OAT received CoQ10 treatment in a double-blind controlled trial. After six months of treatment, the CoQ10 content in the ejaculate of patients receiving CoQ10 increased and sperm motility also improved. Six spontaneous pregnancies occurred in the CoQ10-treated patient group, while three spontaneous pregnancies occurred in the placebo group. Thakur et al.[45] reported that 150 mg CoQ10 daily administration improved semen parameters in oligospermic men.

Zn is the most abundant metal in the body after iron. Zn is known as a metal that plays an important role in testicular development and sperm maturation. Studies have shown that Zn supplementation has a protective effect on spermatozoa against oxidized thiol levels and therefore may improve impaired semen function. Alsalman et al.[46] reported that oxidized thiol levels and semen returned to normal in a study of 60 infertile men receiving 220 mg Zn sulfate daily for three months.

Low sperm Zn concentrations are associated with low sperm fertilization capacity. Ebisch et al.[47] reported on men taking 26 mg Zn and 66 mg folic acid for five weeks. These authors reported an improvement in sperm concentration. However, they did not observe any improvement in other sperm parameters. In contrast to baseline, a positive correlation was found between motility, serum sperm concentration, inhibin B levels and Zn.

Selenium has been shown to be an essential trace element for testosterone biosynthesis and sperm formation.[48]

N-acetylcysteine (NAC) is a naturally occurring compound. It is a reaction product of the amino acid L-cysteine and functions as a precursor of GPx. Randomized clinical trials have reported that selenium supplementation, alone or in combination, improved sperm count, motility and morphology, and sperm concentration in men suffering from infertility.[49,50] Safarinejad and Safarinejad.[49] studied the effect of selenium and NAC in 468 infertile men with idiopathic OAT. The patients were followed for a period of 30 weeks. Serum follicle stimulating hormone (FSH) decreased, while serum testosterone and inhibin B levels increased. As a result, all semen parameters improved in the treated population. In addition, selenium plus NAC administration significantly improved semen parameters.

Paradiso Galatioto et al.[51] reported a study to determine the effectiveness of antioxidant supplementation on semen parameter quality and natural pregnancies in men with infertility, six months after retrograde varicocele treatment. Twenty men with varicocele received antioxidant supplementation treatment: NAC and a cocktail of vitamins and minerals (vitamin E, vitamin C, vitamin A, thiamine, biotin, B12, riboflavin, magnesium, Fe, Cu, manganese, Zn). A significant increase in sperm count was reported in the treated population. However, no significant association was found between multiple supplement treatment and other sperm parameters, including morphology and motility.

Tremellen et al.[52] reported a prospective, double-blind, randomized, placebo-controlled study involving 60 couples with infertility. Patients were randomly assigned to a treatment group receiving a capsule containing 6 mg Lycopene, 500 mg vitamin C, 400 IU vitamin E, 26 micrograms Se, 25 mg Zn, 5 mg folate and 1000 mg garlic or placebo daily for three months. The antioxidant group showed a significant improvement in successful pregnancy rate (38.5%) compared to the control group (16%). There was no significant difference in oocyte fertilization rate and embryo quality between the two groups.

Sengupta et al.[53] 2022 reported that intense antioxidant supplementation may lead to excessive reducing substances and negatively affect fertility by disrupting key oxidation mechanisms in homeostasis.

Sadeghi et al.[54] 2022 also focused on the balance between oxidative and reductive stress in terms of sperm integrity. According to their research, intense supplementation may increase reductive stress, which leads to impaired sperm function and therefore may be counterproductive.

Oxidative Stress and Antioxidant Capacity in the Female Reproductive System

Each month, a group of oocytes begins to grow and develop in the ovary, but in only one, the dominant oocyte, meiosis I continues. This process is determined by an increase in ROS and is inhibited by antioxidants. In contrast, the progression of meiosis II is enhanced by antioxidants[23], suggesting a complex relationship between ROS and antioxidant homeostasis in the ovary. The increase in steroid production in the growing follicle causes an increase in cytochrome P450, leading to ROS production. ROS produced by the oocyte before ovulation are suggested to be important inducers of ovulation.[2] O_2 deficiency stimulates follicular angiogenesis, which is important for adequate growth and development of the ovarian follicle. Follicular ROS promote apoptosis, whereas GSH and follicular FSH balance this effect in the growing follicle. Estrogen increases in response to FSH, triggering CAT formation in the dominant follicle and thus preventing apoptosis.[23]

ROS have both negative and positive effects on mammalian ovaries.[27] ROS cause many different physiological and

pathological activities in the ovaries from oocyte maturation to fertilization. In cyclic oocytes, different markers of OS are negatively affected.[55] ROS concentrations may also play an important role in implantation and fertilization of oocytes, and a related study described the localization of SOD in the ovary and reported that Cu-Zn SOD was localized in the granulosa cell of growing follicles and mature Graafian follicles, while manganese SOD (MnSOD) was localized in the luteal cells of the corpus luteum in rats.[23]

A rapid decrease in progesterone is required for adequate follicle development in the next cycle. Cu-Zn SOD increases in the corpus luteum during the early and mid-luteal phase and decreases during the regression phase. This activity parallels the change in progesterone concentration, in contrast to the increased lipid peroxide levels during the regression phase.

Other possible explanations for the decrease in Cu-Zn SOD are increased prostaglandin (PG) F2-alpha or macrophage activity or decreased blood flow in the ovary.[23] PG F2-alpha induces SO anion production by luteal cells and phagocytic leukocytes in the corpus luteum. Reduced blood flow in the ovary causes tissue damage through ROS production. During regression, Mn SOD concentrations in the corpus luteum increase to scavenge ROS produced in the mitochondria by inflammatory reactions and cytokines. Complete breakdown of the corpus luteum causes a significant decrease in Mn SOD in the regressing cell. At this point, cell death is inevitable.[25] Cu-Zn SOD is closely associated with progesterone production, while Mn SOD protects luteal cells from OS-induced inflammation.[23]

OS affects the entire reproductive process throughout a woman's life. ROS attack the eighth carbon atom of guanine in DNA, producing 8-hydroxy-deoxyguanosine (8-OHDG), an oxidized deoxyguanosine which is present in higher concentrations in aging oocytes.[56] 8-OHDG is the best known base modification in mutagenic damage. 8-OHDG causes base mutation and mismatches in DNA replication and leads to the conversion of G mutations to T and G:C to T:A. Therefore, 8-OHDG has become a biomarker for OS.

OS is implicated in many female diseases, including PCOS, the most common endocrine abnormality in women of reproductive age, with a prevalence of approximately 18%. PCOS is characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovaries.[57] Several studies have shown the presence of OS in PCOS patients. In a study by Costello et al.[58] increased serum prolidase activity, as well as higher total oxidant status and OS indices, the ratio of oxidants to total antioxidant status (TAS), were found in PCOS patients. Decreased mitochondrial O_2 consumption and GSH levels, together with increased ROS production, may explain mitochondrial dysfunction in PCOS patients. Physiological hyperglycemia produces increased levels of ROS from mononuclear cells, which activates the release of the cytokine tumor necrosis factor-α (TNF-α) and increases the inflammatory transcription factor nuclear factor-kappa B (NF-κB). As a result, TNF-α concentrations, a known mediator of insulin resistance, are further increased. The resulting OS creates an inflammatory environment that further promotes insulin resistance.[58]

Bremer and Miller.[59] reported that disruption of the delicate balance between intra- and extra-ovarian factors interferes with mature oocyte formation and leads to infertility. Furthermore, insulin plays a special role in PCOS and *in vitro* studies have reported that insulin stimulates proliferation of protozoa, increases secretion of androgens mediated by LH and increases cytochrome P450 expression of the LH and IGF-1 receptor.[59] Cytochrome P450 is weel known to be able to increase ROS concentrations. This evidence suggests that ROS participate in the pathological process of PCOS.

Endometriosis is a benign, estrogen-dependent, chronic gynecological disease characterized by the presence of endometrial tissue outside the uterus. Lesions are usually found on dependent surfaces in the pelvis. They can also be found in other sites, such as the intra-abdominal organs, lungs and urinary tract. Endometriosis affects 6% to 10% of women of reproductive age and is also known to be associated with pelvic pain and infertility[60], but it is a complex and multicomponent disease that cannot be explained by a factor but is the result of a multifactorial pathogenetic mechanisms. These include retrograde menstruation, impaired immunologic response, genetic predisposition and inflammatory components.[61] The most likely mechanism explaining pelvic endometriosis is the retrograde menstruation and implantation theory. This theory suggests that the reflux of endometrial tissue from the fallopian tubes during menstruation explains its extra-tubal localization and adhesion to the pelvic viscera.[62]

Studies have reported mixed results regarding the detection of OS markers in patients with endometriosis. Some studies have not reported increased OS in the peritoneal fluid or circulation of patients with endometriosis[63], while others have reported increased levels of OS markers in those with the disease. Proinflammatory and chemotactic cytokines play a central role in the recruitment and activation of phagocytic cells, which are a major source of both ROS and RN.[64]

2010 measured *in vivo* peritoneal fluid and plasma 8-iso-PGF2-alpha levels in patients with endometriosis. They found that 8-iso-PGF2-alpha levels in both urine and peritoneal fluid of patients with endometriosis were significantly higher compared to control measurements.[65] 8-iso-PGF2-alpha levels are likely to be useful in predicting oxidative status in diseases, such as endometriosis, and may also be helpful in determining the cause of concurrent infertility.

Patients with endometriosis are more likely to have lower pregnancy rates compared to those without endometrosis. In addition to spermatotoxic peritoneal fluid, ROS may mediate poor oocyte and embryo quality and may also contribute to the subfertility experienced by patients with endometriosis.[66] The peritoneal fluid of women with endometriosis contains low concentrations of the antioxidants vitamin C[64] and GPx.[67] The decrease in GPx levels is thought to be due to a reduced response of endometrial cells to progesterone.[68]

Unexplained infertility is defined as the inability to conceive after 12 months of unprotected intercourse in couples where known causes of infertility have been assessed and no impairments have been identified. It is therefore considered a diagnosis of exclusion. Its pathophysiology remains

unclear, but the literature suggests a possible contribution of increased levels of ROS, indicated by increased levels of the lipid peroxidation marker malondialdehyde (MDA), especially compared to the concentration of antioxidants in the peritoneal cavity.[69] Increased amounts of ROS in these patients suggest a decrease in antioxidant defenses, including GSH and vitamin E. The low antioxidant status of peritoneal fluid may be a determining factor in the pathogenesis of idiopathic infertility.

Folate (vitamin B9) is considered indispensable for reproduction. It is involved in amino acid metabolism and methylation of proteins, lipids and nucleic acids. Acquired or inherited folate deficiency contributes to homocysteine accumulation. Recently, Altmäe et al.[70] reported that the most important variation in terms of effect on folate metabolism is the *methyl-tetra-hydrofolate reductase (MTHFR)* gene polymorphism 677C/T.

Pregnancy is a state of OS caused by increased metabolic activity in placental mitochondria and increased ROS production due to the higher metabolic demands of the growing fetus.[71] SO anions produced by placental mitochondria are thought to be the main source of ROS and lipid peroxidation contributing to OS in the placenta and are supported by mitochondrial production of lipid peroxides, free radicals and vitamin E in the placenta, which increases as the pregnancy progresses.[72] In the second trimester, the placenta gradually matures and increases in size with less hair and larger blood vessels. The cytotrophoblast becomes a single cell and gradually replaces the endothelial layer lining the smooth muscle of the spiral artery. Gradually, maternal blood penetrates from the mother's spiral artery into the interstitial space.[72] In this process, placental tissue produces a large amount of free radicals and generates oxidation. Intense stress occurs, but the placenta gradually adapts to this environment and returns to normal through appropriate antioxidant activity.[73,74] While physiological concentrations of endogenous glucocorticoids maintain fetal development, excess glucocorticoids *in utero* (i.e., maternal stress) adversely affect mammalian offspring primarily by "programming" abnormalities that occur after birth.[75] ROS are also thought to play a role in different phases of the endometrial cycle. The late luteal phase is characterized by high levels of lipid peroxide and a decrease in the antioxidant SOD. ROS affect PGF2α secretion through NF-κB activation.[76] Decreased estrogen and progesterone levels lead to decreased SOD expression and thus OS in the uterus, ultimately causing endometrial shedding and lack of implantation.

OS leads to endothelial cell dysfunction. Endothelial cell dysfunction in the uterus is the cause of many diseases. Moreover, there are many causes of endothelial cell dysfunction. TNF-α is known to cause endothelial cell damage, but the antioxidant cytokine Mn SOD neutralizes SO anions produced by TNF-α. This process is a self-defensive mechanism against TNF-α-induced OS. In addition, defective placentation causes placental hypoxia and ischemia-induced reperfusion injury, and the resulting OS triggers cytokine and PG release, leading to endothelial cell dysfunction and playing an important role in the development of preeclampsia.[77] Furthermore, ROS generated from NADP(H) oxidase are critical for vascular endothelial growth factor signaling *in vitro* and angiogenesis *in vivo*.[78] Endothelial NADP(H) oxidase activated by growth factors and cytokines produces ROS in a non-excessive amount. ROS produced in and around the vascular endothelium may play a role in normal cellular signaling mechanisms.

Preeclampsia is a vascular disorder of pregnancy, usually involving impaired placental development. Normotensive women also present with a complex multisystem disorder. OS increases nitration of p38 MAPK and consequently decreases its catalytic activity, which may cause the poor implantation and growth restriction observed in preeclampsia. Increased ROS concentrations in preeclampsia patients have been suggested by increased levels of MDA, a marker of lipid peroxidation.[79] Under normal conditions, the disruption of circulatory homeostasis is mainly due to vascular endothelial dysfunction in pre-eclampsia. It is characterized by a tendency to cause vasoconstriction and low anticoagulant activity. ROS appear to play a critical role in the endothelial dysfunction associated with preeclampsia. In other words, the pathological event in preeclampsia is OS-regulated vascular endothelial injury due to increased placental ROS or decreased antioxidant activity.[80]

Autoantibodies against the angiotensin receptor, AT1, particularly the second loop (AT1-AA), may stimulate NAD(P)H oxidase, leading to increased ROS production. In preeclamptic women, the AT1 receptor has been reported to increase both SO anion production and overexpression of NAD(P)H oxidase in cultured trophoblasts and smooth muscle cells. Thus, early placental development may be affected by dysregulated vascular development and function due to altered gene expression mediated by NAD(P)H oxidase.[81] Preeclamptic women produce excess ROS and show higher NAD(P)H expression than those without the pre-eclampsia. More specifically, women with early-onset pre-eclampsia have been reported to produce greater amounts of SO anions than women with late-onset disease. Affected women also have reduced TAS and placental GPx and low levels of vitamin C and E. Deficient vitamin C intake appears to be associated with an increased risk of pre-eclampsia and some studies have shown that pre-pregnancy multivitamin supplementation may reduce the risk of pre-eclampsia in normal or underweight women.[82]

CONCLUSION

OS affects the woman's entire reproductive system and process. Excessive production of ROS leads to OS events. ROS, including superoxide (O_2- -), H_2O_2 and hydroxyl (-OH), cause DNA damage, lipid peroxidation and protein damage. When mild OS, such as SOD and GPx, are formed, the antioxidant system starts to work. Moreover, when ROS levels exceed the scavenging capacity of the system, the redox system can also repair oxidized and damaged molecules using NADPH as the original electron source. Therefore, maintaining a high redox potential is an absolute precondition for maintaining reproductive systems in a healthy state.[10]

In this review, we mainly discussed the related reproductive diseases caused by OS and a series of signaling pathways, including PCOS, endometriosis, and preeclampsia. OS during

reproduction activates many molecules, but the interaction between them is not very clear, which requires us to identify signaling cues in other organs or other diseases. Compared to other diseases, research into diseases of the reproductive system is extremely complex, especially in both male and female infertility and pregnant women.

In this discussion, we summarized the following. First, OS plays a role in the development of reproductive system diseases, despite also being critical at low levels for normal fertility. Second, OS greatly disrupts reproductive organs, including the placenta. Third, the inflammatory environment caused by OS induces a series of signaling activations in the uterus.

Finally, by speculating on the relationship between these signaling molecules, we can re-examine future development trends in reproductive system diseases. In the future, a strategy to strengthen antioxidant systems and target mitochondria would be a big step. To increase the body's antioxidant capacity, ROS production from the mitochondrial ETC, which occurs in response to high glucose and fatty acid levels, should be reduced and also this reduction of ROS production should not significantly affecting ATP production.

Targeting mitochondria and increasing the overall antioxidant defense system may be critical. It is now well recognized that the pharmacological effects of antioxidants depend on their targeting. It is also known that it is beneficial to use classes of antioxidants separately from each other. Appropriately powered and well-designed randomized, placebo-controlled trials are needed to evaluate any evidence for the benefits or harm of a range of antioxidants at different concentrations and locations, or both.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.G., S.H.K., Concept: H.G., S.H.K., Design: H.G., S.H.K., Data Collection And Processing: H.G., S.H.K., Analysis And Interpretation: H.G., S.H.K., Literature Search: H.G., S.H.K., Writing: H.G., S.H.K.

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