Emerging Role of Oxidative Stress in Female Reproduction

Alka B. Patil, Sarika P. Mitkare, Vilyesh R. Ghetia

Abstract

The oxygen atmosphere under which we live produces a continuing barrage of oxidative damage to all biomolecules. The same reactions creating life may also be responsible for many pathologies in female reproduction due to free radicals. Oxidative stress is a state characterized by an imbalance between pro-oxidant molecules including reactive oxygen species and reactive nitrogen species and anti oxidant defences .Oxidative stress play a key role in female infertility, natural and assisted. Oxidative stress influences the entire reproductive life span of a woman. Imbalance between pro-oxidant and anti oxidant can lead to a no. of reproductive diseases such as endometriosis, polycystic ovary disease, unexplained infertility. Pregnancy complication such as spontaneous abortion, recurrent pregnancy loss, pre eclampsia can develop in response to oxidative stress. The interventional strategies to overcome oxidative stress are highlighted.

Keywords: Oxidative stress; Free radical; Reactive oxygen species; Reactive nitrogen species; Antioxidant; Infertility; ART.

A.C.P.M Medical College, Dhule- 424001

Alka B. Patil, Prof & HOD, Obst & Gynaecology

A.C.P.M Medical College, Dhule- 424001

Sarika P. Mitkare, Senior Resident, Dept. of Obst & Gynaec

A.C.P.M. Medical College, Dhule-424001

Vilyesh R. Ghetia, Resident, Dept. of Obst & Gynaec

Introduction

Human body has evolved mechanism to prevent damage by means of multifaceated antioxidant systems. Understanding the role of oxidative stress in this context is a multidisciplinary topic that requires the integration of knowledge from different sources. Free radicals and related species have attracted a great deal of attention in recent years. They are generated in our body by various endogenous systems, exposure to different physiochemical conditions or pathological states.[1]

Oxidative stress results from an imbalance between pro-oxidant (free radical species) and the body's scavenging ability (antioxidant). ROS are a double-edged sword; they serve as key signal molecules in pathological process involving the female reproductive tract but also can serve as key signal molecules in physiological process.[2] Antioxidants, capable of neutralizing free radicals or their action act at different stages. They act at the level of prevention, interception and repair. All the biological molecules present in our body are at increased risk of being attacked by free radicals. Such damaged molecules can impair cell function and even lead to cell-death, eventually leading to disease state.

We review female infertility related to antioxidant defences and oxidative stress; examine potential sources of oxidative stress from ovarian germ cell through the conception. This article focuses on evaluating the current evidence of oxidative stress in the normal functioning of the reproductive tract and assessing their in role in infertility and assisted reproduction. The review highlights the role of oxidative stress in early pregnancy loss.

Correspondence to:
Dr.(Mrs.)Alka B Patil , Email : alkabpatil@rediffmail.com

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We capture the oxidative stress links in the chain of events leading to endometriosis and polycystic ovarian diseases. We address the role of antioxidants in overcoming oxidative stress. Interventional strategies to overcome oxidative stress are highlighted.

The purpose of this review is to summarize recent literature, examining the possible role of free radicals in female reproduction, and future prospects. We performed a literature search in PUBMED, using the keywords oxidative stress, free radicals, reactive oxidative stress, reactive nitrogen species, infertility to explore the subjects.

Free Radicals (ROS and NOS) and Their Physiological Action

Recently there is a growing evidence of possible role of highly reactive products of oxygen, termed free radicals, in infertility. A free radical is defined as molecular species capable of independent existence and containing one or more unpaired electrons, making them paramagnetic and relatively active. These are formed as natural byproducts of oxygen metabolism. When out of control, they become toxic and start damaging body tissues by a process called oxidative stress.[3] Free radical species are highly reactive and unstable, only becoming stables by acquiring electrons from, lipids, proteins, nucleic acids, carbohydrates or any nearby molecule and thus causing a cascade of damage and disease.[2] Free radicals are very short lived, with half lives in milli, micro or nanoseconds.

Free radicals are capable of causing cells to lose their structure, function and can eventually destroy them. They are continuously produced by our body's use of oxygen such as in respiration and some cell mediated immune functions. They are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air pollution, pesticides, etc. (Li and Trush, 1994). There are two key types of free radical species: Reactive oxygen species and reactive nitrogen species. ROS, such as super oxide anion (O^{-2})hydrogen peroxide (H_2O_2), and the hydroxyl radical($OH^{\bullet}$), are generated from molecular oxygen.[4]

Reactive Oxidative Species

The superoxide radical is formed when electrons leak from the electron transport chain. [5] The dismutation of superoxide results in the formation of hydrogen peroxide. The hydroxyl ion is highly reactive,causes strand breaks resulting in DNA damage. Some oxidase enzymes can directly generate the hydrogen peroxide radical. ROS have been implicated in more than 100 diseases. They have a physiological and pathological role in the female reproductive tract.

ROS may act as important mediators in hormone signaling, oocyte maturation, ovarian steroidogenesis, ovulation,corpus luteal formation, luteolysis, luteal maintenance in pregnancy, implantation, blastocyst development.[5]

The main radicals in the ROS are the superoxide radical, hydrogen peroxide, hydroxyl and the singlet oxygen radicals. An array of protective mechanisms can neutralize these oxidants or free radicals. Non-enzymatic antioxidants are Vitamin C, taurine, hypotaurine and glutathione: these protect against extraneous sources of ROS. The enzymatic antioxidants include Superoxide dismutase, catalase, glutathione peroxidase and glutaredoxin.[4]

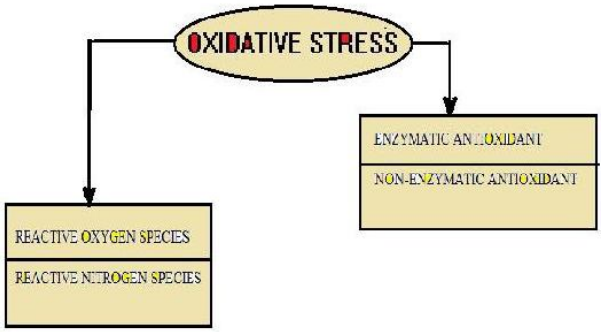
Reactive Nitrogen species

Nitric oxide (NO) is synthesized during the enzymatic conversion of L-arginine to Lcitrulline by nitric oxide synthase (NOS). With an unpaired electron, NO, which is a highly reactive free radical, damages proteins, carbohydrates, nucleotides and lipids and, together with other inflammatory mediators; results in cell and tissue damage; low-grade, sterile inflammation and adhesions.[5]

Beneficial Role of Free Radicals

It has to be emphasized that ROS and RNS are both produced in a well regulated manner

Figure 1: Oxidative Stress Occurs When the Balance Between Highly Reactive Radicals (Oxidants) and Antioxidants Tips Towards the Oxidants; It Negatively Contributes to Reproductive Processes



to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. Most cells can produce superoxide (O_2^-), hydrogen peroxide (H_2O_2) and nitric oxide (NO) on demand. Hence, it is worth emphasizing the important beneficial role of free radicals.

1. Generation of ATP (universal energy currency) from ADP in the mitochondria: oxidative phosphorylation
2. Detoxification of xenobiotics by Cytochrome P450 (oxidizing enzymes)
3. Apoptosis of defective cells
4. Killing of micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes.
5. Oxygenases (eg. COX: cyclo-oxygenases, LOX: lipoxygenase) for the generation of prostaglandins and leukotrienes, which have many regulatory functions.

In recent years, it has become increasingly clear the ROS, such as O_2^- and H_2O_2 may act as second messengers.[1]

Is a given fact that perfectly normal and healthy individuals, even under basal conditions, produce ROS through their aerobic metabolism. Therefore cells have developed a wide range of antioxidant mechanisms to limit the production of ROS, inactivate them and repair cell damage.[5]

Oxidative Stress In female Reproduction

Oxidative stress (OS) is caused by an imbalance between pro-oxidants and antioxidants. This ratio can be altered by increased levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), or a decrease in antioxidant defense mechanisms. Excessive ROS production may overpower the body's natural antioxidant defense system, creating an environment unsuitable for normal female physiological reactions. This, in turn, can lead to a number of reproductive disorders including endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility.[5]

ROS and the Follicles

Oxidative stress plays a role in the physiology of ovarian function. The follicular fluid microenvironment is a biological window into the quality of the oocyte and the subsequent embryo that is generated. Phagocytic macrophages, parenchymal steroidogenic cells and endothelial cells generate ROS in the ovaries (Halliwell and Gutteridge, 1988). Different investigators have found that ROS are involved in folliculogenesis, follicle maturation, ovulation and corpus luteal function. Cells involved in steroidogenesis such as theca cells, granulosa lutein cells, and hilus cells show stronger oxidative enzyme activity (Scully and Cohen, 1964).

The follicular fluid microenvironment is important for follicular maturation and granulosa cell functions. The expression of Cu-Zn-SOD and Mn-SOD is closely related to steroidogenesis in the human ovary.[6] The follicular fluid environment surrounding the oocytes may play a critical role in fertilization and embryo development, influencing IVF outcome parameters such as fertilization, embryo cleavage, and pregnancy rates.[7]

ROS and Corpus Luteum

Antioxidant enzymes such as Superoxide dismutase (SOD) are expressed cyclically in steroid-producing cells. ROS therefore plays a role in the formation of the corpus luteum and steroidogenesis. Cu-Zn-SOD expression peaks from the early to mid-secretory phase, and this parallels progesterone production by the corpus luteum. Thus, Cu–Zn-SOD has a protective role in the maintenance of the corpus luteum (Sugino *et al*, 1996).Cytokines influence the induction of Mn-SOD.[6]

ROS and Fallopian Tube

Several studies demonstrated the presence of cytokines, prostaglandins, metabolites of lipid peroxidation and ROS in fluid samples of fallopain tube (Tamate *et al*, 1985). The equilibrium of these components serves as an optimal milieu for fertilization and the transport of the pre embryo. An endogenous nitrogen monoxide system exists in the fallopian tubes. Nitric oxide has a relaxing effect on smooth muscle and it has similar effects on tubular contractility. Deficiency of NO may lead to tubal motility dysfunction, resulting in retention of the ovum, delayed sperm transport and infertility. Increased NO levels in the fallopian tubes are cytotoxic to the invading microbes and also may be toxic to spermatozoa (Rosselli *et al*, 1995), leading to infetility.[8]

ROS and Menstrual Cycle

Cyclical variations in the expression of superoxide dismutase in the endometrium have been demonstrated. Superoxide dismutase is an enzyme involved in scavenging the superoxide radical and protecting the cells from oxygen radical toxicity. Endometrial changes in the proliferative and secretory phase during each cycle are influenced by various antioxidants expressed in the endometrium. Production of prostaglandin F2α (PGF2α) in endometrium is stimulated by ROS through cyclooxygenase (COX) in human endometrial stromal cells. PGF2α level is maximum at menstruation and is responsible for endometrial shedding. Thus a close interaction was hypothesized among SOD, ROS and PGF2α resulting in endometrial shedding at menstruation.[4]

Reactive Nitrogen species

NO synthesis increases with follicular development.[9] Nitric oxide radical is one of the local factors involved in ovarian follicullogeneis and steroidogenesis. Nitric oxide acts through activation of various iron containing enzymes. Plasma concentrations of nitrate monitored during the follicular cycle, have revealed peak levels at ovulation. The presence of endothelial NO synthase in human corpora lutea and the expression has been reported in the mid and early luteal phase and to a lesser extent in the late luteal. Follicular fluid NO levels are altered in patients with infertility associated diseases. Some studies have demonstrated the relationship between NO concentrations in follicular growth and programmed follicular cell death (apoptosis). [5]

Antioxidants

'Antioxidants' are substances that neutralize free radicals or their actions (Sies, 1996).Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals.[10] *There are two types of antioxidants in* human body: enzymatic anti oxidants and nonenzymatic anti-oxidants.

Enzymatic antioxidants are also known as natural antioxidants, they neutralize excessive ROS and prevent it from damaging the cellular structure.[5] Endogenous antioxidants are of two types, low-molecular-weight compounds, like uric acid, bilirubin, thiols, and coenzyme Q10, and larger molecular enzymes, like catalase, superoxide dismutase, and glutathione peroxidase glutathione reductase, beta carotene, and carotene.[10]

Antioxidants may prevent and/or improve different diseased states (Knight, 2000). Zinc is an essential trace element, being a co factor for about 200 human enzymes, including the

cytoplasmic antioxidant Cu Zn SOD, isoenzyme of SOD mainly present in cytosol. Selenium is also an essential trace element and a co factor for glutathione peroxidase. Vitamin E and tocotrienols are efficient lipid soluble antioxidants that function as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including LDL.[11] Vitamin C is a chain breaking antioxidant that stops the propagation of the peroxidative process. Vitamin C also helps recycle oxidized vitamin E and glutathione.[5]

Reproductive Diseases

Infertility

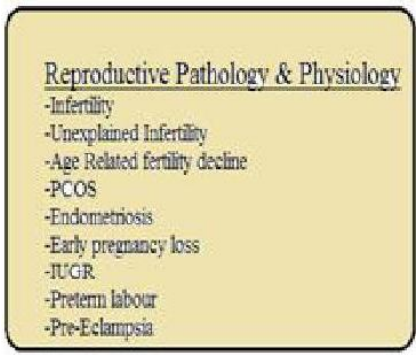
Oxidative stress affects both natural and assisted fertility. Oxidative stress biomarker have been found in various physiological functions. Other studies have suggested that ROS are involved in various causative factors of infertility (e.g. peritoneal factor, tubal factor, endometriosis and unexplained infertility). The tubal and peritoneal microenvironments influence fertilization and early embryonic development.

Oocyte and spermatozoa can experience direct damage; ROS also induce lipid peroxidations with related effects in cell's division, metabolite transport and mitochondrial dysfunction. Impaired fertilization may be due to an environment of oxidative stress in the peritoneal cavity. Even when fertilization occurs,apoptosis leading to embryo fragmentation, implantation failure, abortion or congenital abnormalities in offspring can occur. Oxidative stress in the fallopian tubes can cause direct adverse effects on the embryo. Concentrations of ROS may play a key role both in the implantation and fertilization of oocytes.[12]

OS and Unexplained Infertility

The pathophysiology of unexplained infertility remains a scientific challenge. Unexplained infertility may be caused by increased generation of ROS in the peritoneal cavity. Women with idiopathic infertility have reduced concentrations of antioxidants and increased ROS induced lipid peroxidation damage resulting in infertility.[6]

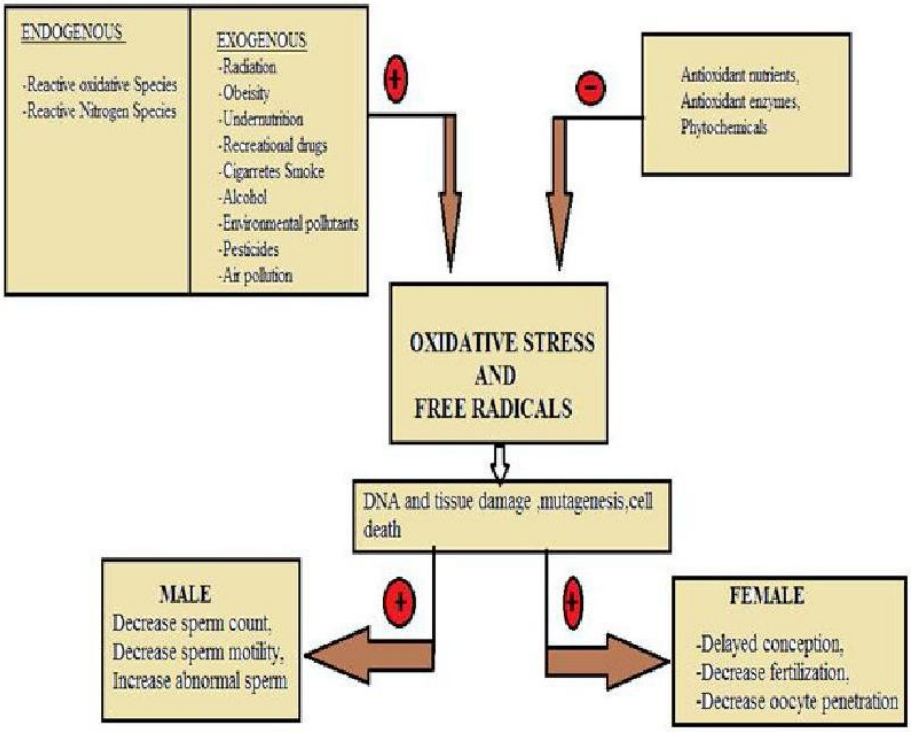
Figure 2: Factors Contributing to the Development of Oxidative Stress and Their Impacts on Female Reproduction



Oxidative Stress and Art

A myriad of physiological and environmental factors are known to influence the success rates of ART in achieving fertilization and pregnancy culminating in living offspring. Reactive oxygen species (ROS) have been widely implicated in the literature to render a significant impact on these outcomes. *In vitro* ART procedures are carried out without the protection of enzymatic antioxidants normally found *in vivo*, leading to unopposed, elevated levels of ROS, which have been shown to adversely affect gametes, gamete interaction, fertilization and pregnancy rates.[13] Several exogenous factors identified in culture media enhance embryo production of ROS: oxygen concentration, the presence of metallic cations, and visible light exposure. Elevated ROS levels generated exogenously or endogenously influence the gametes, gamete interaction, fertilization and pregnancy rates with IVF/ICSI. Oxidative insult to embryos can lead to 2- cell block, embryonic arrest or even embryonic demise. Higher levels of ROS in the follicular fluid and semen are associated with poor fertility outcomes with assisted reproduction. Oxygen (O_2) tension in healthy oviducts and uteri is normally 2% - 8% in the presence of gametes. Elevated O_2 levels (>20%) may create unfavorable conditions that produce excess

Figure 3: Role of Oxidative Stress in Fertility



free oxygen radicals. These radicals may then damage lipids, DNA, and proteins, as well as block embryo development. [10]

Age-Related Fertility

Oocyte quality decreases in relation to increasing maternal age. Recent studies have shown that low quality oocytes contain increased mtDNA damage and chromosomal aneuploidy, secondary to age-related dysfunctions. These mitochondrial changes may arise from excessive ROS. SOD and glutathione peroxidase expression decrease in ovary from premenopausal to menopausal period. ROS may play a role in age related decrease in estrogen production. Higher level of oxidative stress have been demonstrated in women of advanced reproductive age undergoing IVF. Free radical induced damage may be implicated, at least partly, for the age related decline in quantity and quality of follicle reserves .Reactive oxidative species pertub the intracellular calcium homeostasis in the oocyte and cause aging of the oocyte.[12]

Oxidative Stress and Endometriosis

Many studies have investigated OS markers and antioxidant status in patients with endometriosis. Inducers of OS may include erythrocytes, apoptotic endometrial cells and undigested endometrial cells in the menstrual effluent. These factors may cause activation and recruitment of mononuclear phagocytes. Activated macrophages induce OS, lipid peroxide formation, and other by-products resulting from the interaction of apolipoproteins with peroxides. OS leads to a localized pelvic inflammatory reaction resulting in increased concentrations of cytokines, growth factors, and other proinflammatory mediators. Ota *et al* (1999) argue that increased OS concentrations are present due to xanthine oxidase concentrations in endometriosis patients. Xanthine oxidase is recognized as an ROS-generating enzyme. Endometrial development affects embryo implantation, and therefore an asynchrony between endometrial receptivity and embryo stage could impair embryo implantation and oxidative stress may result in this asynchrony. [14]

Oxidative Stress and PCOD

Polycystic ovary syndrome is also associated with decreased antioxidant concentrations, and is thus considered an oxidative state. The mononuclear cells of women with PCOS are increased in inflammatory state , which occurs more so from a heightened response to hyperglycemia and C-reactive protein (CRP). Hyperglycemia generates increased levels of ROS from mononuclear cells, which then activate the release of TNF-alpha. As a result, concentrations of TNF-alpha, a known mediator of insulin resistance, are further increased. The resultant OS creates an inflammatory environment that further increases insulin resistance and contributes to hyperandrogenism.[12] Oxidative stress seems to be involved in altered steroidogenesis in the ovaries, thus contributing to increased androgen production, disturbed follicular development and ultimately, infertility.[15]

Placental Oxidative Stress

Normal human placentation is determined for the most part by the proper invasion of the uterine spiral arteries by a genomically normal trophoblast. This invasion governs the changes in the anatomy of the placental vasculature to ensure optimum perfusion by the maternal vessels. Definite metabolic changes occur in embryos during the transition from first to second trimester. During the period of embryonic organogenesis, the revailing oxygen tension is low. Thus, the production of ROS perhaps is reduced to prevent DNA damage induced by oxidants. At the end of the first trimester, a definite rise in oxygen tension occurs in the intervillous space from less than 20 mm Hg to 50 mm Hg, leading to a burst in oxidative stress. Lower oxygen tension in the first trimester stimulates the invasive capacity of the trophoblast. This is probably due to increased activity of integrins that help trophoblast cells to proliferate.[16] Implantation is a very well orchestrated dialogue between the embryo and the uterine environment. A burst of OS occurs with the establishment of maternal circulation (Jauniaux *et al*, 2000). Deficient trophoblastic invasion results in an impaired dialogue between the embryo and its environment and is associated with abortion.[6]

The antioxidant enzymes form other protective system, playing a key role in the response of trophoblast to the burst of perfusion by maternal blood. With the increase of oxygen saturation and oxidative stress, the placenta employs a number of physiologic adaptations (Burton, 2009). Levels and activity of antioxidant enzymes: catalase, glutathione peroxidase, manganese and cooper, zinc superoxide dismutase are increased within placental tissues. This response is evolved as a defense mechanism to reduce harm to placental tissues exposed to this burst of oxidative stress.[8]

Interventional Strategies to Overcome Oxidative Stress

Oxidative stress appears to be due to increased generation of ROS rather than a depletion of antioxidants. It is important to identify the source of increased ROS generation. The underlying etiological factor should be determined. Patients with history of smoking should be advised to stop smoking. Any exposure to drugs, toxic substances and radiation should be checked and patients advised to stop exposure to them. Infections of the reproductive tract should be treated with appropriate antibiotics. Initially, specific therapeutic options directed against the etiological cause of raised ROS should be tried. Antioxidants can be started directly when a specific etiology cannot be identified (idiopathic infertility).[17]

The use of both natural and synthetic antioxidants in treating infertility patients is currently under investigation.[9] Antioxidants including Vitamins A, C, and E, zinc, glutathione, beta-carotene, and carotene are dietary supplements that aid the female body's defense system. Glutathione is localized in tubal fluid and the oocyte itself. This nonenzymatic antioxidant promotes zygote development.[10] Systemic supplementation with vitamin C has been used in patients who are infertile, in those with luteal phase defects and in those who have experienced recurrent

abortions. Supplementation with 400 mg of vitamin C improved ovulation induction rates with clomiphene (Igarashi, 1977). Vitamin C may play a role in fertilization (Wilson, 1973). [6] Antioxidant mimetic molecules are also in the developmental stages and include phenolic, porphyrinic, and peptidylic structures of Zn, Cu and Mn complexes that mimic SOD.[10] In female reproduction folate plays an important role in oocyte quality and maturation, implantation, placentation, organ development and fetal growth.Women receiving folic acid supplement had, oocytes of higher quality and a higher degree of maturation than those not receiving supplements. Further, the levels of homocysteine which correlates negatively with oocyte quality was significantly lower in follicular fluid in women on folic acid supplements while undergoing ART. Zinc posses antioxidant and anti apoptotic properties that counteract ROS. Antioxidants such as vitamin E (α tocopherol), vitaminC (ascorbic acid) and vitamin A help maintain oxidant-antioxidant balance in tissues.[4]

Antioxidant principles from natural resources possess multifacetedness in their multitude and magnitude of activities and provide enormous scope in correcting the imbalance. Therefore, much attention is being directed to harness and harvest the antioxidant principles from natural resources.[18] Large no. of epidemiological studies have suggested that some pathologies can be prevented or delayed to some extent by dietary changes such as increase consumption of fruits,grains and vegetables.These protective effect is reasonably ascrised to high level of anti oxidant (vit. E, vit. C, carotenoids, polyphenol etc.) containt in this kind of food.[19]

Literature supports that antioxidant supplementation can result in generation of better quality embryos in ART. Supplementation of the media with optimal concentration of antioxidants, amino acids and vitamins C, E results in scavenging of the free radicals. Supplementation of culture media with antioxidants; disulphide reducing agents or divalent chelators of cations is beneficial for embryos cultured in vitro. Enhanced embryo survival and blastulation rates have been reported with antioxidant supplementation of the media. Antioxidants such as Ethylene diaminetetraacetic acid (EDTA), low oxygen tension, superoxide dismutase can be utilized in overcoming OS in the ART setting. Antioxidants are used as media supplements for various sperm preparation techniques. Pentoxifylline, glutathione and albumin are effective in reducing ROS levels, when used in sperm preparation media. Sperm preparation helps reduce the exposure of the functional spermatozoa to the defective spermatozoa and leucocytes.[16] Besides supplementation, modifying the embryo environment by eliminating ROS producing sources such as light and high oxygen concentrations can improve culture conditions. Modification such as utilizing lower oxygen tension (5%) has shown benefit in human embryos and resultant improved implantation rates. Although an imbalance between the harmful ROS and buffering antioxidants seem to explain the gamete and embryo related factors that lead to low ART success rates, the translation of this knowledge to modulate culture or oocyte and embryo handling techniques needs further research.[4]

Supplementation antioxidant therapy may be of benefit to the patients with early pregnancy loss; During preconception and early stages of conception. Evidence of increased teratogenecity associated with high dose. Vit. A exist, but normal vitamin consumption may not be harmful.

Discussion

Oxidative stress in gynaecological environment is likely to be an important mediator of conception. Evaluation of the impact of oxidative stress on women's fertility and early pregnancy loss represents a significant gap in our knowledge about reproduction and suggest, new research in this area is warranted. Oxidative stress develops when the generation of R.O.S overwhelms the scavenging capacity of anti-oxidants.

Oxidative stress is involved in pathophysiology of infertility, assisted fertility and female reproduction. Infertility is a significant health problem and diagnosis and treatment are stressful, invasive and costly. The role of oxidative stress in female infertility is an understudied and compelling area for investigation. Identifying modifiable factors to decrease oxidative stress in gynecological environment may be an inexpensive and non invasive therapy for increasing fertility. Antioxidants may be advised when specific etiology cannot be identified as in idiopathic infertility.

ART are being increasingly used to help infertile couples realize their dream of having a biological child: strategies have been designed to surround oxidative stress based on understanding the effects of ROS on various stages of fertilization. Management option for overcoming oxidative stress methods include in vivo and vitro anti-oxidant supplementation. Research still needs to be performed that will minimize oxidative stress during ART and focus on creating media as close as possible to the physiological milieu in vitro to improve effectiveness and outcome.

Anti-oxidant supplementation, immunomodulators and selective progesterone receptor modulator (SPRM) with anti-oxidant effects have been investigated as possible treatment for endometriosis, but compelling evidence on benefits of various modalaties is lacking.

The establishment of pregnancy requires a harmonic hormonal ovarian and fallopian tube function, a receptive uterus able to respond a variety of biochemical and molecular signals produced by developing conceptus as well as specific interactions between endometrium and extra embryonic membrane.[10] A new understanding of early materno-fetal relationship has emerged and with it new insight in to the pathogenesis of these disorders, unifying the two, is the concepts of placental Oxidative stress. Evaluation of environmental factors effect on oxidative stress molecular pathways can serve possible solutions for female reproduction, malformation. The imbalance of oxidative agents and antioxidant have been proposed as causative factors in IUGR, gestational DM, pre- eclampsia.[8]

Reference values for ROS and NOS, minimum safe concentration and physiologically beneficial concentration have yet not been defined. Measurement of oxidative stress in vivo is controversial. The sensitivity and specificity of various oxidative stress marker is not known. Measurement of biomarkers of oxidative stress is subject to interlaboratory variations and interobserver differences. A uniform method with comprehensive assessment of oxidative stress biomarkers should be used so result can be compared acrossed the studies. Parallel identification and isolation of anti oxidant principle from natural sources are simultaneously presenting enormous scope for their better therapeutic application.[20]

As a part of healthy life style; well balanced, wholesome diet, antioxidant supplementation is recognized as an important means of improving free radical protection. The traditional Indian diet, spices and medical plants are rich sources of natural anti-oxidants. Higher intake of foods with functional attributes including high level of anti-oxidants in functional foods is one strategy that is gaining acceptance.

Newer and future approaches include, gene therapy to produce more antioxidants in the body, genetically engineered plant products with high level of antioxidant, synthetic anti oxidant enzymes (SOD mimic), novel biomolecules and the use of functional food enriched with antioxidant. Co-ordinated research involving biomedical scientist, nutritionist and gynecologist can make significant difference to human health in the coming decades. Research on free radicals and anti-oxidants involving these is one such effort in the right direction.

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