Review Article

Oxidative Stress and its Role in Female Infertility and Assisted Reproduction: Clinical Implications

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Abstract

Reactive oxygen species (ROS) are involved in physiological functions and act as mediators in various signaling processes. Elevated or sustained generation of free radicals and non radical species derived from free radicals can lead to an imbalance in the intracellular redox homeostasis. Normally, any excess levels of reactive radical and nonradical species generated are intercepted by antioxidants. An excess of the free radicals however, can precipitate pathologies in the female reproductive tract. Oxidative stress (OS) is involved in various pathological conditions such as abortions, preeclampsia, hydatidiform mole, fetal teratogenecity, preterm labor and intrauterine growth retardation, all of which lead to an immense burden of maternal and fetal, morbidity and mortality. In addition evidence suggests that oxidative stress plays a role in the proinflammatory changes seen with polycystic ovarian disease and also in the pathogenesis of endometriosis and tubal factor infertility. Our review captures the role of OS in assisted reproduction specifically in *in vitro* fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) and *in vitro* maturation of oocytes (IVM). We also examine the role antioxidants play in modifying the fertility outcomes with assisted reproductive techniques. Finally *in vivo* and in vitro strategies to modulate the influence of ROS and establish an optimal redox state are also discussed.

Keywords: Reactive Oxygen Species, Oxidative Stress, Antioxidants, Infertility, Assisted Reproductive Techniques

Introduction

There is a complex interaction, of free radicals and antioxidants, which modulates the generation of oxidative stress. Free radicals may be defined as any species with one or more unpaired electrons in the outer orbit (1). Electrons within atoms and molecules occupy regions of space known as orbital. Each orbital can hold a maximum of two electrons. A single electron in the orbital is said to be unpaired (2). Generation of the highly reactive free radicals is an inherent feature of normal cellular metabolism. As more and more research is focusing on the reactive oxygen species, there is new light being shed on the role of these radicals in physiological functions and whenever there is an excess of the free radicals, they precipitate pathologies in the female reproductive tract.

The main radicals in the ROS are the superoxide radical, hydrogen peroxide, hydroxyl and the singlet oxygen radicals (Fig 1). 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 SOD, catalase, glutathione peroxidase and glutaredoxin (Fig 2).

Oxygen radicals and ROS play both a physiologic and pathologic role in the female reproductive tract. Normally the pathologic effects are exerted by various mechanisms including lipid damage, inhibition of protein synthesis, and depletion of ATP. Studies have examined how free radicals affect a gamut of physiologic functions in female reproduction such as are oocyte maturation, ovarian steroidogenesis, ovulation, implantation, and formation of fluid filled cavity, blastocyst, luteolysis and luteal maintenance in pregnancy.

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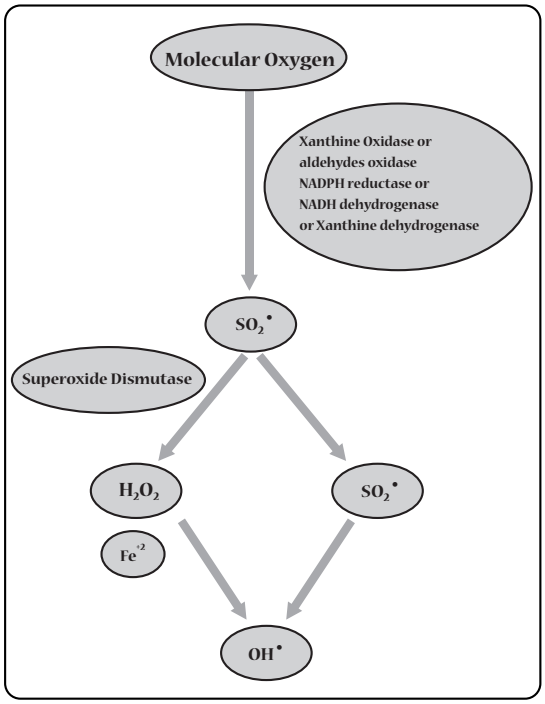

Fig 1: Redox pathway of superoxide generation

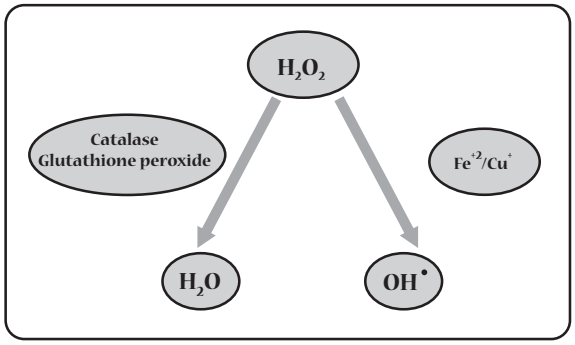

Fig 2: The haber-weiss reaction

Reactive oxygen species, reactive nitrogen species, exogenous and endogenous antioxidants

a) Reactive oxygen species

Ros or free radicals are powerful oxidants that are oxygen derivatives. In the male and female reproductive tract, ROS, such as super oxide anion (O^{-}_2) hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH.), are generated from molecular oxygen (3). Reactive nitrogen species are also included in free radical species.

ROS generation is mainly due to electron leakage from the mitochondrial membrane (4). Low levels of ROS are required to maintain normal cellular redox homeostasis (5). OS occurs when the production of ROS increases or when levels of antioxidants decrease. The unstable ROS having unpaired electrons, causing cell damage by attacking biomolecules like lipids, proteins and nucleic acids (3). Normal cellular aerobic metabolism also results in the generation of ROS. Minimal levels of ROS act through signaling pathways, which is necessary for normal physiological functions in the female reproductive tract (6, 7). ROS not only affects processes such as oocytes maturation, fertilization and embryo development (7) but also pregnancy (8). Studies have also documented OS in the pathophysiology of preeclampsia, hydatidiform mole, teratogenic defects and spontaneous abortions. Literature has documented role of OS in the pathophysiology of infertility and assisted fertility (9, 10).

Superoxide ions are highly reactive and cause tremendous intracellular damage. They are produced by neutrophils and cytochrome P450. Several cell structures such as membranes, proteins, enzymes and nucleic acids more prone to damage by superoxide ions. Since the hydroxyl ion- a derivative of H_2O_2 involved in apoptotic cell damage and hydroperoxyl radical, the conjugated acid of superoxide anion can initiate lipid peroxidation chain reaction (Fig 3) in membrane lipids (6, 7).

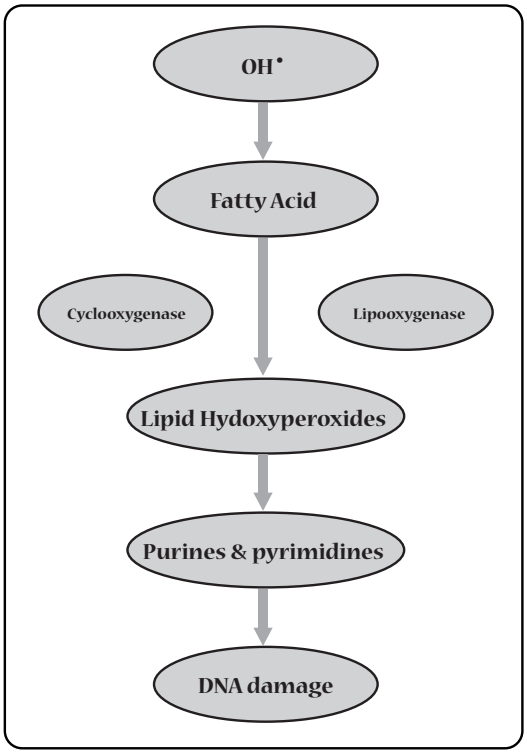

Fig 3: Induction of DNA damage by free radicals

b) Reactive nitrogen species

L-arginine (L-Arg) along with the molecular oxygen forms nitric oxide (NO) and citrulline and this reaction is catalyzed by nitric oxide synthase (11)

(Fig 4). Nicotinamide adenine dinucleotide dihydrophosphate (NADPH) and tetrohydrobiopterin (H4B) are utilized in the reaction. Due to its unpaired electron, NO is highly reactive free radical and induces adverse alterations in the structure of proteins, carbohydrates, nucleotides and lipids. It also has a role in cell and tissue destruction, sterile inflammation and formation of adhesions (11). Although NO is involved in the regulation of various physiological processes, and can prove to be toxic if present in excess levels (11-13), the actions of NO in a cell depend on its concentration, the cellular redox state, and the abundance of metals, protein thiols and low-molecular weight thiols (glutathione) as well as other nucleophil targets (14). NO potently relaxes arterial and venous smooth muscles and, less strongly, inhibits platelet aggregation and adhesion formation.

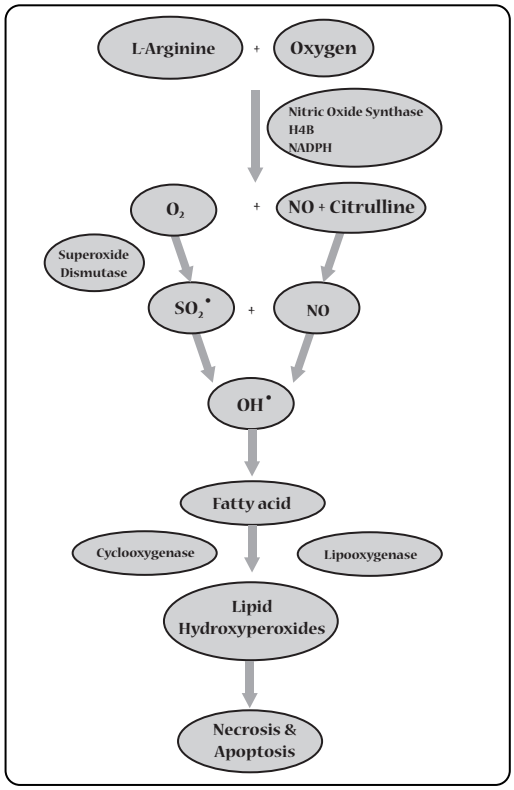

Fig 4: Apoptotic induction by ROS and NOS

c) Antioxidants

ROS can be neutralized by enzymatic and non enzymatic antioxidant defense system to protect the cell. Therefore antioxidants can be described as a substance, which can dramatically delay or prevent oxidation of an oxidizable substrate.

Enzymatic antioxidant consists of catalase, SOD and glutathione peroxidase/glutathione reductase; the non enzymatic antioxidants are vitamin C, vitamin E, vitamin A, pyruvate, glutathione, taurine and hypotaurine (5, 15). Usually a balance is maintained between pro-oxidants and antioxidants in a cellular homeostasis but any change in the ratio like shift towards pro-oxidants can cause oxidative stress. Enzymatic and non enzymatic antioxidants are abundantly present in both male and female reproductive tract. Enzymatic antioxidants such as SOD are localized in cytoplasmic (Cu, Zn-SOD), mitochondrial (Mn-SOD) and endometrial glandular cells (16, 17). Non enzymatic antioxidants such as vitamin C and E are present in the ovary while carotene and ascorbate reside in follicular fluids (18). The propagation of oxidative stress can be prevented by chain breaking property of antioxidants like vitamins C and E (4).

Antioxidants can also be classified as exogenous or endogenous. Exogenous antioxidants come from the regular dietary intake and include vitamins A, E and C along with others such as alpha lipoic acid, selenium, Co-enzyme Q10 (CoQ10), grape seed contains proanthocyanidins, pycnogenol and zinc (3). 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 (3, 19).

Nonenzymatic antioxidants (NAE) glutathione (GSH) a substrate of glutathione peroxidase (GPX), play a role in oocytes and embryos to prevent DNA damage and enhance embryonic protection (20). The antioxidative action of hypotaurine neutralizes hydroxyl radicals and prevents lipid peroxidation. Taurine, a by product of hypotaurine, neutralizes end-products of the peroxidation cascade reaction, that is cytotoxic aldehydes and thus protects cells from hazardous effects of ROS. The release of hypotaurine and taurine can be induced by ascorbate, a potent direct antioxidant. Ascorbic acid also prevents DNA damage. In this way, taurine, hypotaurine and vitamin C which are abundantly found in tubal and follicular fluid, play a vital role in antioxidant protection of gametes and embryos (21, 22). NADPH oxidase-mediated generation of superoxide anion is inhibited by vitamin E. Oral antioxidants vitamins can neutralize the superoxide anion, hydrogen peroxide, and hydroxyl radicals and reduce the potential damage to membrane lipid and DNA (23).

Enzymatic antioxidants located in the cystol such as copper, zinc, superoxide dismutase (Cu, Zn-SOD) and manganese superoxide dismutase (Mn-SOD) located in mitochondria allow superoxide radical to be scavenged (24). Both these enzymes protect the tissues against toxic oxygen radical. Either catalase

or glutathione peroxidase (GPX) scavenges hydrogen peroxide, a by-product of SOD action (refer Fig 2). A pivotal role is played by GPX as it reduces the generation of lipid hydroperoxides and hydrogen peroxide and confers embryo protection and improves oocytes maturation (25).

Overview of oxidative stress

ROS and antioxidants remain in balance in normal physiological conditions. When the balance is disrupted towards an elevated level of ROS, OS occurs. Unrelenting and elevated production of ROS is more often the cause of OS and this can overwhelm the antioxidant reserves. Cells have developed a wide range of antioxidants systems to limit production of ROS, inactivate them and repair cell damage Age-related decline in fertility has been suggested to be induced by free radical damage (26). ROS plays a role during pregnancy (27) and normal parturition (28, 29) and in initiation of preterm labor (30, 31). Repeated ovulation induces free radical mediated damage to the ovarian surface epithelium and chronic inflammation leading to development of epithelial ovarian cancer (32, 33). Ovulation-induced oxidative base damage and damage to DNA of the ovarian epithelium can be prevented by antioxidants, especially in women who are genetically at high risk for ovarian cancer (34). ROS cause detrimental changes to various macromolecules initiating lipid damage, inhibition of protein synthesis, and depletion of ATP (35). Oocyte maturation, ovarian steroidogenesis, ovulation, implantation, formation of blastocyst, luteolysis and luteal maintenance in pregnancy are physiological processes modulated by ROS (36-41).

ROS are key messengers in maintaining physiological functions in the female reproductive tract. However excessive and unrelenting ROS generation causes various pathologies in the female reproductive tract. Redox balance and homeostasis is maintained by the presence of ample amounts of antioxidants, measuring levels of the antioxidants, individually or as total antioxidant capacity (TAC), has also been examined (36, 37, 42-44).

There are dynamic changes in the antioxidant expression profiles of oocytes at different stages such as germinal vesicle and metaphase II stages as well as in the oviducts and reproductive tract secretions in many of the studies on human and animal tissues (16). Extensive literature highlights the role of OS in the physiological functions in female reproduction and also in disease states such as pre-eclampsia (45, 46), hydatidiform mole (47-49), free radical-induced birth defects (50), polycystic ovarian disease and other situations such as abortions (8).

Measurement of oxidative stress

The presence of ROS and antioxidants in the female reproductive tract has been demonstrated by various methodologies in studies conducted both in animal and human reproductive fluid and tissue. The basal levels of intracellular ROS in the leukocytes in whole blood and embryos can be determined by utilizing the dihydroethidium and dichlorodihydrofluorescein-diacetate probe. Dihydroethidium is oxidized to ethidium and is a measure of superoxide anion. Dichlorodihydrofluorescein-diacetate is oxidized to dichlorofluorescein and dihydrorhodamine, which measures hydrogen peroxide, peroxynitrite and hypochlorous acid, respectively (51, 52). Electron paramagnetic spin trap resonance (EPR) is the most direct method to measure free radicals such as superoxide. Chemiluminescence technique for precise assessment of ROS levels has been reviewed by our group (15). A number of OS biomarkers have been investigated including superoxide dismutase, glutathione peroxidase, conjugated dienes, lipid peroxides, thiobarbituric acid reactive substances, glutaredoxin, oxidative DNA adducts, NO and TAC (16, 37, 38, 40, 53-55). Enzyme linked immunabsorbant assay (ELISA) has been utilized to measure concentration of SOD, catalase, GpX in the follicular and granulosa cell culture media (56). Metabolites of NO (nitrite and nitrate) can be measured by a method using nitrate reductase and the Griess reaction (57). Spectrophotometric measurement of NO and its metabolites can be made in peritoneal fluid and tissue culture supernatants (58). Total NO (nitrite and nitrate) levels in the serum and follicular fluid assay of NO are measured via a rapid-response chemiluminescence's analyzer (53). Oxidative stress biomarkers have been determined in the placenta by immunohistochemistry or western blot analysis. Oxidative DNA adducts 8-hydroxy 2- deoxyguanosine-have been studied by immunostaining in cumulus and mural granulosa cells (59).

Oxidative stress and the 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 (3). The levels of SOD increase and the ROS levels increase in the endometrium in the late secretory phase just before menstruation indicating that these changes in the level of expression indicate in-

volvement in endometrial breakdown and shedding (60). The differential expression of the antioxidants indicates the existence of oxidative stress during cyclical endometrial changes. Estrogen- progesterone withdrawal led to increased expression of COX-2 mRNA and increased prostaglandin F2α synthesis in endometrial cells, cultured *in vitro*. These effects were proposed to be ROS mediated nuclear factor kappa B (NFκB) activation (61, 62).

It was also mentioned that production of prostaglandin F2α (PGF2α) in endometrium is stimulated by ROS through cyclooxygenase (COX) in human endometrial stromal cells (ESC) *in vitro*. 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. In conclusion, estrogen-progesterone withdrawal causes cyclooxygenase-2 (COX-2) expression stimulation and PGF2α synthesis via ROS mediated nuclear factor kappa B (NFκB) activation (60, 61). In a study it was demonstrated that NFκB is involved in manganese superoxide induction by TNFα or protein kinase C in human endometrial stromal cell (ESC). This mechanism was hypothesized to be a self defense system of ESC against TNFα –mediated oxidative stress (60).

Users of long term progesterone only contraceptives ones are reported to experience abnormal and excessive uterine bleeding. It was revealed that women experiencing excessive bleeding had elevated mean levels of serum NO metabolites compared with women with normal bleeding (57) Literature reports point towards abnormal angiogenesis induced as a result of oxidative stress as a causative factor of the abnormal bleeding (63).

Role of oxidative stress in pregnancy

In the past 10 years or so, researchers have attempted to answer an important question: does OS play a key role in pregnancy outcomes? There are few literature reports which analyze a direct relationship between ROS level and pregnancy outcome.

Higher levels of superoxide are reported to be generated from the placental mitochondria (27, 64). Elevated levels of lipid peroxides and vitamin E have been reported in pregnancy (65, 66). The antioxidant and the peroxidation product levels are increased in pregnancy (67, 68). During the third trimester of pregnancy, levels of intracellular ROS in the leucocytes increase. Pregnancy leads to leucocyte activation and these results in an inflammatory state (52, 69). The leukocyte activation response is further exacerbated in pregnancies complicated by preeclampsia. Lipid peroxides levels are increased during the second trimester and then it tapers off, lowering further after delivery (65). Aberrant placentation causes placental ischemia which leads to generation of placental oxidative stress. Placental oxidative stress has a key role in the pathophysiology of spontaneous abortions, preeclampsia, and in pregnancies complicated by intrauterine growth restriction (IUGR) (70, 71).

Oxidative stress at parturition

Elevated OS has been reported with both vaginal and operative deliveries but there is no consensus in the literature on whether vaginal mode or cesarean section is associated with higher levels of OS. The lack of consensus could be because of the different oxidative stress markers assessed. Mocatta et al reported that vaginal mode of delivery was associated with higher levels of OS as reflected by greater levels of MDA compared with cesarean section (29). Term labor triggers a compensatory elevation of the nonenzymatic antioxidant reserves in the fetal red blood cell compartment (72). This protects the neonate from the hyperoxia encountered at birth. Nonenzymatic antioxidant reserves have been found to be diminished in preterm neonates. There is a greater susceptibility in preterm neonates for free radical-induced damage such as retinopathy and bronchopulmonary dysplasia (73-75). Uncomplicated term labor, term intrauterine growth retardation (76) and pre-eclampsia were associated with elevated cord blood malondialdehyde (77) levels, a marker of oxidative stress (29, 78, 79). The presence of elevated levels of cord blood MDA in neonates born via vaginal delivery and in those born to mothers with pre-eclampsia is reflective of oxidative stress.

A randomized controlled trial found that cord blood protein carbonyl levels--a biochemical marker of oxidative stress–were significantly lower in preterm infants than in term infants. The lower protein carbonyls in very low birth weight infants (VLBW, <1500 gms) may be due to localized oxidative stress (e.g., in the lung or retina).

Role of oxidative stress in preterm labor

Prematurity is an important cause of neonatal mortality and long-term morbidity such as neurodevelopmental delays, blindness and cerebral palsy. The production of reactive oxygen species, prostaglandins, proinflammatory cytokines and proteases has been implicated in the initiation of term and preterm labor (80).

Chorioamniotis is commonly associated with preterm labor. Oxidative stress plays a role in the etiology of chorioamniotis (CAM). In CAM, there is

increased NADP (H) oxidase- activity-a ROS generating enzyme. CAM is a leading cause of preterm labor and causes the up regulation of COX-2 (cyclooxygenase-2) enzyme in the placenta and prostaglandin synthesis (81). One study reported that 4 hydroxynenal was associated with increased expression of COX-2 and prostaglandin E2 in the placenta. 4 hydroxynenal is a marker of oxidative stress.
Metalloproteinase are a group of endopeptidase enzymes with a collagenolytic activity and are activated in preterm premature rupture of membranes. The redox balance determines the matrix metalloproteinase activity of the amniochorionic membranes (82). Metalloproteinase activity was found to be increased directly by superoxide anion, a byproduct of macrophages and neutrophils (82).

Oxidative stress and neonatal outcomes
Elevated oxidative stress has been reported in term infants with fetal distress and in preterm infants. Preterm infants are highly susceptible to free radical damage because of low antioxidant reserves. Preterm infants are exposed to hyperoxia and this leads to free radical mediated disorders (75), including bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP) and hypoxic ischemic encephalopathy (73, 74). Both enzymatic and nonenzymatic scavenging antioxidants are deficient in preterm infants (83). The best approach to preventing the free radical-induced injuries in preterm infants is by providing optimum oxygen therapy and avoiding hyperoxia.
OS adversely affects fetal outcomes in preterm infants, and in term infants, it is associated with fetal distress. Significantly elevated concentrations of 8-iso-prostaglandins F2α, a product of lipid peroxidation, were detected in cord blood from singleton pregnancies complicated by moderate or thick meconium-stained liquor (84). Management of oxidative stress with amnioinfusion may be beneficial in patients with meconium stained liquor because it lowers lipid peroxide levels, a marker of oxidative stress.
Oxidative stress may have a role in programming embryo and fetal development in-utero. Studies have investigated utilizing scavenging antioxidants in neutralizing the OS. Studies in mice have shown that the antioxidant ά-tocopherol decreased the occurrence of drugs and hyperglycemia induced neural tube defects.

Oxidative stress and diabetic pregnancy
Diabetes complicating pregnancy is associated with elevated levels of OS and reduction in antioxidant reserves and this may lead to poor fetal outcomes. Elevated concentrations of thiobarbituric acid reactive substances (TBARS) and reduced antioxidant enzymes (i.e. Copper zinc superoxide dismutase [Cu ZnSOD]), catalase and glutathione peroxidase were demonstrated in hemolysed erythrocytes from patients with pregnancies that were complicated with well-controlled diabetes (85). The oxidative stress parameters, TBARS and antioxidant capacity were measured in all three trimesters of pregnancy (85, 86). A large study on 70 patients with pregestational diabetes demonstrated an association between poor fetal outcomes and elevated peroxide levels and reduced antioxidant capacity (87).
In a semi-randomized controlled trial on antioxidant-supplementation during pregnancy, sister chromatid exchange (SCE) rate was used as an indicator of DNA damage. The test group that was given the multivitamin/mineral supplementation showed a decrease in SCE rates after 10 weeks of supplementation (88). Park et al demonstrated benefits of antioxidant supplementation resulting in reduced DNA damage. Excellent glycemic control during pregnancy may help with reduction of the OS levels in pregnancy complicated with diabetes.

Implication of oxidative stress in recurrent pregnancy loss
RPL has been reported to affect around 1%-5% of couples attempting pregnancy (89, 90). In the reproductive age group of women, that is 15-45 years of age, loss of three or more consecutive pregnancy before 20 weeks of gestation or weighing less than 500 g is considered recurrent pregnancy loss (91, 92). The majority of the pregnancy losses are early, usually before 12 weeks of gestation (91). There is a body of literature highlighting the various risk factors and etiological factors such as demographic factors including age and racial/ethnic differences, cytogenetic abnormalities, anatomic abnormalities of uterus and others. Besides these are autoimmune diseases, infections, environmental factors and occupational exposures (91, 92) responsible for spontaneous or recurrent abortions. In about fifty to sixty percent of miscarriages etiology remains unknown, while endothelial damage, impaired placental vascularisation, and immune malfunction are some of the proposed factors to play a role in idiopathic abortion (92). Dysfunctional placentation may be a common underlying factor in the pathophysiology of a continuum of spectrum of disorders like abortions, preeclampsia and intrauterine growth restriction.
Maternal circulation is established by the end of the first trimester of pregnancy and at this point in time there is an oxidative stress burst within the placenta

(93). Abnormal placenta due to various causes leads to oxidative stress and syncytiotrophoblast dysfunction which ultimately leads to pregnancy loss (94). Leucocytes show similar changes in pregnancy as they produce during sepsis. In early stages of pregnancy increase in leukocyte count is observed, causing increased production of superoxide radical (69, 95). Elevated generation of ROS has been demonstrated by chemiluminescence technique in patients with recurrent pregnancy loss (96). A study demonstrated that level of activation of respiratory burst produced by opsonized zymosan and by isolated granulocytes in response to N-formyl-methionyl-leucyl-phenyl-alanine showed significantly different outcomes. The differences observed were due to the oxidase activity of inhibitor of tyrosine protein kinases, protein phosphates and inhibitor of nitrogen-activated proteinkinase p38 MAPK in the women with recurrent abortions when compared to women with normal reproductive functions. This study concluded that females with habitual abortions are more predisposed to oxidative stress (96).

In pregnant women significant elevation of lipid peroxides and plasma glutathione and lower levels of vitamin E, vitamin C and beta–carotene are reported to be associated with recurrent miscarriages (97, 98). Similarly lower concentration of α - tocopherol, total thiols and glutathione are observed in patients with unexplained abortions (99).

A study has reported evidence of higher prevalence of hyperhomocysteinemia in women with recurrent pregnancy loss (RPL), although little is known about its pathophysiology (100). Defective chorionic villous vascularisation is a proposed hypothesis by which increased homocysteine concentration is linked to RPL. Folic acid and vitamin B6 both have a role in the metabolism of the amino acid homocysteine. Folic acid and vitamin B6 supplementation may be a preventative measure in lowering the elevated homocysteine levels (101).

Impact of OS in various gynecological conditions and female infertility

Reactive oxygen species include oxygen derived radicals as well as non radical oxidants. The role of ROS and female infertility has been a subject of considerable interest and research over the last decade (102). Reactive oxygen species and antioxidants appear to have a physiological role in reproductive processes including oocyte maturation, fertilization, luteal regression, and endometrial shedding, (54, 103). Macrophages, neutrophils and granulosa cells in Graffian follicles are a source of ROS which are balanced by antioxidants. During follicular maturation oocytes are well protected against the toxic injury due to oxidative stress by important antioxidants such as catalase, superoxide dismutase (SOD), glutathione transferase, paraoxanase, heat shock protein 27 and protein isomerase (104). However an imbalance between proxidants and antioxidants has been postulated in female infertility as it may affect ovulation, fertilization, embryo development and implantation (3). Reactive oxygen species are a double-edged sword, which have been localized in the female reproductive tract in various animal and human studies. The localization of ROS and antioxidants in the ovary, fallopian tube and endometrium and the role of ROS in modulating the cyclical ovarian and endometrial functions have been discussed in the preceding parts of the review. There is some understanding of the role of oxidative stress in infertility and its causative factors like, endometriosis, unexplained infertility and tubal factor infertility (11, 105-107). Peritoneal macrophages are activated in patients with endometriosis, which is associated with increased scavenger receptor activity (108). The activated macrophages are a source of increased ROS generation in patients with endometriosis (109).

OS has been suggested to be causative in etiologies such as endometriosis, tubal, peritoneal and unexplained infertility and even polycystic ovarian syndrome (PCOS) (11, 106, 108, 110, 111). The aberrations in the tubal, peritoneal or endometrial environment which result in infertility are modulated by the generation of excess proxidants as evident by raised levels of ROS from the fluid evaluation.

Patients with hydrosalpinges undergoing *in vitro* fertilization-embryo transfer (IVF-ET) have lower implantation and pregnancy rates. The exact mechanism by which hydrosalpingeal fluid (HSF) induces embryo toxicity is not clearly defined. Negative influence of HSF may be due to embryo toxic substances in the fluid (112, 113). OS mediated embryo damage has been postulated as one of the reasons. (5). Bedaiwy et al measured ROS, total antioxidant capacity and lipid peroxidation (LPO) from HSF at laparoscopic salpingectomy. Further 2-cell murine embryos were incubated with HSF to correlate OS biomarkers and blastocyst development. While ROS was detected in 45% samples, there was a higher blastocyst development when ROS was detected in HSF in comparison to HSF samples devoid of ROS. This was possibly because lower concentration of ROS beneath a threshold harmful to embryos represents normal ROS generated by functional endosalpinx. HSF with non detectable ROS represented fluid derived after extensive endosalpingeal damage. Detection of ROS at low concentration may be a marker of normal tubal

secretory function. There was a negative correlation between increasing HSF concentration and blastocyst development rate, suggesting the possible role of OS in the embryo toxicity of HSF.

Endometriosis is one of the most enigmatic diseases with multiple theories as regards etiopathogenesis. Despite extensive research, the exact mechanism of infertility related to endometriosis is controversial. There is increased turnover of macrophages in the peritoneal cavity of women with endometriosis. Activated macrophages in the peritoneal environment have been suggested as a source of ROS (114) and evidence of increased generation of ROS has been demonstrated by higher levels various OS biomarkers in women with endometriosis .

OS is responsible for the localized pelvic inflammatory reactions with increased concentrations of cytokines, growth factors, and other proinflammatory mediators. Wang et al reported higher concentration of ROS, using chemiluminescence's analysis for peritoneal fluid from women with endometriosis and unexplained infertility in comparison to PF from tubal ligation controls (115). This increase in ROS was not significant, perhaps because PF ROS may not be directly responsible for infertility in these women. Other markers of lipid preoxidation such as antibodies to oxidized low density lipoproteins have been reportedly elevated in women with endometriosis (116). Evidence of raised OS biomarkers in endometriotic lesions including 8-OH-deoxyguanosine (8-OH-dG) and lipoperoxide contents as compared to normal endometrial tissue further strengthens the role of OS in endometriosis (117). Elevated lipoprotein complexes and lowered antioxidants contribute to excessive growth of endometrial stromal cells implicating the role of OS in infertility associated with endometriosis (118). Szczpanska et al detected significantly lower levels of superoxide dismutase and glutathione peroxidase in PF of women with endometriosis as compared to those with idiopathic infertility (114). Both SOD and glutathione peroxidases are potent antioxidants with ability to prevent adverse effects of OS by inhibiting generation of hydroxyl radical. In contrast to this study, Polak et al and Ishikawa et al did not find any difference in SOD and glutathione peroxidase activity in PF from women with endometriosis or idiopathic infertility (36, 111). This obvious discrepancy between studies measuring OS biomarkers could be because OS occurs locally at the site of bleeding and may not result in an increase in total PF concentration of OS biomarkers. Whether levels of antioxidant enzymes and ROS influence fertility remains to be confirmed through further studies, they seem to play a role in the development and progression of the disease.

Unexplained infertility is a diagnosis of exclusion when no cause is detected on routine fertility workup. However research has revealed high levels ROS in peritoneal fluid specimens from women with unexplained infertility in contrast to fertile controls undergoing tubal ligation (115). Malondialdehyde, a lipoperoxidation end product was found in higher concentrations in PF from women with unexplained infertility (119). Even levels of antioxidants seem to be lower in these women as compared to levels in PF from fertile women (111).

Polycystic ovary disease affects 5-10% of women in the reproductive age. The current understanding is that PCOS is not only a gynecological condition but a metabolic syndrome with associated disorders such as insulin resistance and dyslipidemia (120). Infertility is related to insulin resistance which disturbs the hormonal milieu in these women. There is emerging evidence now that chronic low inflammation is often present in women with PCOS with the possible role of increased OS. Elevated levels of oxidized proteins (protein MDA) and antiendometrial antibodies have been detected in sera of women with PCOS as compared to controls (121). Further elevated ROS are being considered responsible for insulin resistance and hyperandrogenism. Gonzalez et al in their study observed that there is generation of ROS from mononuclear cells which occurred in response to hyperglycemia in women with PCOS. This increase in ROS was seen both in obese and lean PCOS when compared to matched controls and was independent of obesity (122). Disturbed OS biomarkers have been observed even in the lean PCOS in yet another by Yilmaz et al where significantly lower antioxidant status was measured in the serum of lean PCOS in comparison to healthy controls. Serum malonyl-dialdehyde, a marker of lipid peroxidation was increased in these subjects. These patients were subsequently treated with metformin or rosiglitazone over 12 weeks and the levels of OS biomarkers in response to medications was assessed. Rosiglitazone seemed to decrease elevated oxidative stress when compared to metformin treatment in lean PCOS. (123). While OS is known to increase in parallel with insulin resistance this study concluded that the decrease in OS levels in lean PCOS treated with rosiglitazone is independent of decrease of insulin resistance. Increased OS in PCOS may explain the increased risk of cardiovascular disease in women with PCOS (124). There has been some recent evidence that women with PCOS have lower levels of glutathione, a powerful antioxidant (125) . Long term risks of ovarian malignancy in women with PCOS have been explored in a recent study. Dinger et al addressed this by ob-

serving an increased DNA damage in the leucocytes with comet assays from women with PCOS. There was also an increased susceptibility to OS induced damage in these women with PCOS (126). OS as is being implicated in the pathogenesis of PCOS may possibly explain the link with long term complications of PCOS including cardiovascular disease and malignancy.

OS and assisted reproduction

Assisted reproductive techniques (ART) have become the treatment of choice in many cases of male and female infertility. Despite technical advances in gamete handling and culture conditions, success rates after ART procedures are still unsatisfactory. Suboptimal oocytes and embryo quality is one of the many reasons contributing to poor pregnancy rates after IVF/ICSI. As our understanding of the metabolism of gametes and embryos has increased over recent years, we have come to hypothesize that OS a negatively impacts human embryo (127) and development (3). *In vivo* human gametes are exposed to physiological levels of ROS and possess natural antioxidant defenses. A physiological amount of ROS in follicular fluid suggests healthy development of oocytes (128). Further an imbalance in the redox state of the developing embryo, as a result of suboptimal culture conditions leads to altered gene expression and impaired adenosine triphosphate generation (129), the latter of which can impair placental and embryo growth (130).

Strategies to overcome OS are aimed at minimizing the exposure of gametes to environments that generate free radicals. Spermatozoa are one of the exogenous sources of ROS. Pasqualotto et al measured the levels of LPO and total antioxidant capacity (TAC) in the follicular fluid from women undergoing IVF treatment, and found both markers correlated positively with pregnancy outcome. They conferred that a certain critical level of oxygen radicals are necessary for oocyte maturation. Further high levels of TAC favorably neutralize the oxidative stress of LPO. This was observed in a similar study by Das et al where follicular fluid from women undergoing IVF was evaluated for OS biomarkers including LPO and TAC (127, 131). Similarly Attaran et al found significantly raised levels of ROS and TAC in the follicular fluid of women undergoing IVF who became pregnant in contrast with those who did not become pregnant. This further proves that a minimal threshold of ROS are indicative of metabolic activity of the follicles (128). However when the levels of various ROS are excessively high around follicular fluid the oocyte quality is comprised resulting in compromised embryos.

TAC was also evaluated by Oyawoye et al in a study of follicular fluid from women undergoing IVF. Mean levels of TAC were significantly higher in the follicles that yielded oocytes that subsequently were fertilized versus those that did not become fertilized (132). These findings corroborate the hypothesis that germinal cells have potent antioxidant defenses that compensate for the increased ROS levels which would otherwise be detrimental for oocyte and then embryo quality. However when the levels of various ROS are excessively high, oocyte quality is comprised resulting in compromised embryos. In a study Seino et al, the effects of OS on the quality of oocytes and embryos in IVF and ICSI cycles was determined by quantitatively measuring levels of 8-hydroxy-2deoxyguanosine (8-OHdG), an indicator of cellular DNA damage in the granulosa cells at oocyte aspiration. Higher levels of 8-OHdG were associated with low fertilization and subsequently low quality embryos. Women with endometriosis had further higher levels of 8-OHdG than did patients with other indications for IVF. Thus accelerated OS induced damage to granulose cells may be responsible for poor outcomes in women with endometriosis undergoing IVF procedures (59).

OS has been implicated in arrest of embryo development and poor quality embryos that develop *in vitro*. Yang et al found higher concentration of intracellular ROS as evident by raised levels of H2O2 from fragmented human embryos in comparison to unfragmented embryos (51). Development of healthy embryos is closely related to and coordinated by the process of oocyte maturation. A study by Bosco et al looked at 63 patients undergoing assisted fertilization by ICSI. Oocytes that failed to fertilize demonstrated apoptosis and increased DNA damage in comparison to control oocytes after pick-up (133). OS is involved in poor development of embryos as evident from declining levels of TAC measured in culture media from poor quality embryos (134). Bedaiwy et al reported that ROS are generated in culture media from developing embryo and are significantly high when there is low fertilization, low cleavage, low blastocyst development and increased fragmentation. This was specific in ICSI cycles as explained by the potential antioxidant activity of cumulus cell mass around oocytes which are denuded during ICSI (135). In a subsequent study the same group identified the role of TAC in growth of human embryos *in vitro*. High levels of TAC were measured in culture media from embryos with high cell numbers, low fragmentation, and blastocyst development after conventional IVF and ICSI. Antioxidants in the culture media seem to neutralize the ROS generated by poor quality em-

bryos and thereby lower TAC levels (135, 136).
The source of ROS responsible for disturbed embryonic growth may be endogenous, generated as a result of embryonic metabolism or exogenous from the culture environment (137). The generation of ROS results from metabolism in oocytes, cumulus cells, spermatozoa or embryos and involves oxidative phosphorylation, enzymes such as NADPH oxidase and xanthine oxidase (138). Oxidative phosphorylation necessarily provides the ATP to meet the energy requirement for the embryo and results in ROS production.
Environmental conditions such as oxygen, visible light, metallic ions, and amine oxidases from dead spermatozoa are sources of exogenous ROS. High O_2 concentration during *in vitro* cultures leads to increased levels of H_2O_2 which cause DNA fragmentation. ROS such as H_2O_2 have been the source of programmed cell death (PCD) or apoptosis which blocks development of embryos to blastocyst(139). Visible light induces ROS production and DNA breakage (140). Therefore transient exposure of embryos to light of ≥5 minutes *in vitro* may cause ROS production (141). *In vivo* oocytes and embryos that are kept in a dark environment with low oxygen tension experience minimal OS related damage.
In vitro culture conditions are unable to mimic the exact physiology *in vivo* as oocytes pass through the tubal and uterine environment with low oxygen tension. Oxygen tension in the oviduct is one quarter to one third of the atmospheric tension. (142). Moreover the follicular antioxidants and the growth factors in tubal and uterine fluid scavenge the excessive ROS produced *in vivo*(7). Since this scavenging system is lacking *in vitro* culture conditions there is bound to be OS related injury and therefore a comparative lag of embryo development quality *in vitro*.
Generation of ROS has been observed during cryopreservation of spermatozoa mainly through lipid peroxidation and membrane lipid damage (143). It is plausible that cryopreservation of embryos may increase rates of lipid peroxidation within the cell membrane due to increase levels of oxygen radicals. Thus such a stress may cause reduced viability of cry preserved embryos. The benefits of adding antioxidants such as ascorbate during cryopreservation of mouse embryo have highlighted the role that free radicals play in cryopreservation related embryo damage (144).

OVS and its impact on IVM

In vitro maturation of the oocyte has emerged over the last decade as a valuable technology in assisted reproduction because of its lower cost, safety and ability to avoid ovarian hyperstimulation syndrome. Many literature reports investigating the nuclear and cytoplasmic maturation kinetics in *in vitro* maturation have reported a relative lag of cytoplasmic maturation from the nuclear maturation (145). Failure of maturation can lead to meiotic arrest (146). The fertility outcomes with IVM remain poor. Oxidative damage to the oocytes in *in vitro* conditions has been proposed as one of the etiological factors of the low success rates with IVM. *In vivo*, oocyte maturation is a complex process which is influenced by the complex interplay of the gonadotropin, the oocyte-cumulus oocyte cell interactions and the enzymatic activities. It is important to mimic the *in vivo* conditions *in vitro*. There is a body of evidence on the various antioxidant supplements and modification of the physical conditions to minimize the ROS generation, the optimum media that will give the best results in terms of *in vitro* maturation, fertilization and pregnancy outcomes is yet to be designed
The follicular fluid environment surrounding the oocyte plays a critical role in fertilization and embryo development, as it can influence both IVM and IVF outcome parameters such as fertilization, embryo cleavage and pregnancy rates (3). *In vitro* culture media is always exposed to oxidative stress due to generation of reactive oxygen species both due to cellular metabolism and external factors influencing the media. Plenty of factors govern the generation of free radicals in the media like oxygen concentration, light, oocyte handling and overall metabolism of the oocytes themselves (7). The lack of proper defense or consumption of antioxidant defense due to generation of ROS in the media allows increased damage to the oocyte DNA, or accelerate apoptosis in an unabated manner. The manipulation of gametes and embryos in an *in vitro* environment when performing IVM carries the risk of exposure of these cells to supraphysiological levels of reactive oxygen species (ROS). The integrity of the antioxidant defenses within the different stages of oocyte may contribute significantly to the overall quality of the oocytes (55, 147, 148). There are literature reports on antioxidant supplementation resulting in better outcomes with IVM. In order to get excellent outcomes of IVM the focus has to be on developing optimal culture system for *in vitro* maturation (145, 149, 150) . It is important that future needs to be directed towards enhancing outcomes with IVM which has higher cost effectiveness and safety when compared with conventional IVF.

Strategies to modulate influence of ROS

OS results from an imbalance between proxidants

and oxidants either due to increase in ROS or lowered antioxidant defenses in the body. Therefore strategies to modulate the OS induced damage should aim to attenuate ROS production or boost antioxidant production. This can be achieved by intercepting formation of ROS, scavenging ROS by antioxidants or utilizing repair mechanism occurring naturally in the body (15). Strategies can affect this in the form of modifications in ART techniques or antioxidant supplements.

Gamete and embryo handling

Spermatozoa, oocytes, their interaction during ART, and embryos are a source of ROS. Therefore strategies to modulate this influence could be effected through modifications in gamete and embryo handling procedures as well. Abnormal spermatozoa and seminal leucocytes are a source of OS and eventually result in poor embryonic growth after insemination at IVF. The swim-up or sperm wash preparation technique results in normal spermatozoa being contaminated with ROS producing abnormal spermatozoa and leucocytes (151). Sperm preparation techniques such as double density gradient centrifugation and glass wool filtration separates leucocytes and immature/dead spermatozoa and thus reduce the ROS induced damage. Prolonged sperm-oocyte incubation (16-20 hours) increases ROS generation. Co-incubation of 1-2 hours results in better quality embryos and improved implantation and pregnancy rates. Prospective randomized trials have recommended shorter sperm-oocyte co-incubation time to improve ART outcomes (152). Mechanical removal of ROS in patients with endometriosis undergoing IVF-ET has been evaluated. Cumulus oophorus rinsing is performed in women with ovarian endometrioma undergoing IVF-ET to improve oocyte and embryo quality (153). This possibly eliminates the effects of high TNF-ά and other cytokines in the peritoneal fluid. Higher levels of OS have been found in granulosa cells from patients with endometriosis and such rinsing procedures may possibly remove the increasing ROS surrounding the aspirated oocytes. ROS are generated intracellularly by the gametes, whether handling them by modified media or mechanical means may completely free the toxic levels of ROS is yet not confirmed. Further studies in these areas may improve our understanding in improvising gamete handling.

Oral antioxidants

Oral antioxidant supplementation is a contentious issue even though many investigators have examined this subject in recent years. While current treatment of subfertile couples is empiric, a recent literature report revealed both positive and negative results with antioxidants therapy (154). Folate plays an important role in DNA synthesis and in spermatogenesis. Folic acid has the ability to scavenge free radicals and therefore is an antioxidant (155). Folate has been shown to increase sperm count, enhance sperm motility and reduces immature cells in semen (156). In female reproduction folate plays an important role in oocyte quality and maturation, implantation, placentation, fetal growth and organ development. 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 (157). Zinc posses antioxidant and anti apoptotic properties that counteract ROS (158). Men with low sperm count ≤20 million per ml had lower concentration of seminal plasma zinc levels than men with normal sperm counts (159). While zinc deficiency is rare in females, animal studies on rabbits suggest that zinc deficiency could affect ovulation (160). Ng et al investigated follicular fluid zinc levels in 33 women undergoing IVF. They found no correlation between zinc levels and presence of oocytes with fertilization rates. This study therefore suggests that zinc content had no bearing with oocyte status or IVF success (161). However what dosage and duration of folic acid and zinc are required to improve fertility outcome needs further research.

Antioxidants such as vitamin E (ά tocopherol), vitamin C (ascorbic acid) and vitamin A help maintain oxidant-antioxidant balance in tissues. Spermatozoa produce ROS through normal physiological process and when imbalanced by normal scavengers perioxidative damage occurs. Antioxidants therefore have positive effect on sperm DNA damage and pregnancy outcome (162). Combined therapy with 400 mg glutathione (GSH), 200 mg vitamin C, and 200 mg vitamin E for 2 months significantly improved sperm concentration and lowered oxidative DNA damage (163). While most studies reported improved pregnancy after treatment of male partners with natural cycles there has been recent report of higher pregnancy after male partners received antioxidants prior to ICSI cycles. In a recent prospective randomized double blind placebo controlled trial Tremellen et al studied antioxidant supplementation using Menevit (combination of lycopene, vitamin E, vitamin C, zinc, selenium, folate, garlic and palm oil) of the male partners of couples undergoing IVF-ICSI. They observed a significant improvement in viable pregnancy rate compared with

the control group (38.5% vs. 18%), who received a placebo (164). Antioxidant supplementation may also have unwarranted adverse effects of desynchronized chromatin condensation, as has been reported by Menezo et al.

The benefits of antioxidants on the female reproductive system though contentious have been reported. In patients undergoing IVF-ET, vitamin supplementation during hormonal stimulation resulted in higher follicular fluid concentration of vitamin C (165). The pregnancy rates though not significantly higher improved in the supplemented group. In a pilot double blind placebo controlled trial study Westphal et al studied the effect of supplements containing vitamin E, iron, zinc, selenium and L –arginine in improving fertility in women. Patients receiving supplement reported a significant increase in ovulation and pregnancy rates compared to placebo (166). In yet another randomized controlled trial supplementation of ascorbic acid (750 mg daily) resulted in significantly higher serum progesterone concentrations and pregnancy rates when compared with the non-supplemented group (167).

Smoking is associated with poor fertilization and lower pregnancy outcome during ART procedures. Follicular fluid levels of ROS were raised in women who had a prolonged history of smoking. The avoidance of smoking definitely helps women as it eliminates smoking-induced OS (168).Antioxidant supplementation can be recommended to this group of women to improve to improve fertility (165)

Optimizing culture conditions and reduction of OS levels in ART

In vitro development of embryos occurs in an environment that mimics the *in vivo* environment but the simulation is not exact. A certain threshold of OS is required for conception to occur *in vivo* (169). *In vitro* manipulation of gametes and embryos favors ROS production and may in part be responsible for lower grade embryos and consequent low pregnancy rates. Reduction of OS leads to improved ART options. Avoiding OS during gamete and embryo culture is complex and options include ROS scavengers. The choice of the appropriate antioxidant and its concentration has yet to be determined. Most of the literature being available through research conducted on murine embryos. Mouse embryos mimic human embryos in their behavior and metabolism in varying culture conditions and this has been exploited in determining the right antioxidant combinations for human culture media as well (170). Sperm preparation media may be supplemented with antioxidants that act as ROS scavengers such as pentoxifylline (171), glutathione (172), N-acetylcysteine (173), and albumin (174). Pentoxifylline scavenge hydroxyl as well as superoxide radicals. Small doses have been beneficial in cryopreserved sperm by inhibiting ROS induced injury (175). Pentoxifylline has been beneficial in reducing H2O2 induced damage in mouse embryos and improving IVF outcome even when added to embryo culture media (176).

Culture media have been supplemented with antioxidant supplements including metal chelators, antioxidant enzymes, thiol compounds, proteins, vitamins and growth factors. Metal ions in culture media can result in ROS production through the Heber–Weiss reaction. When added to culture media metal chelators such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid when added to culture media improved development to the blastocyst stage in murine and bovine embryo. (177, 178). Thiol compounds such as GSH, β-mercatoethanol, and cysteine in culture media were observed to improve murine and bovine embryo production through antioxidant effect (179, 180). Supplementing with antioxidant, such as ascorbic and vitamin E have been promising, though the exact concentrations need to be elaborated through future research.

Olson et al supplemented culture media for bovine embryos with vitamin E (100 μM), vitamin C (100 μM), and a combination of both to confirm their benefit in improving embryo development. The number of expanded blastocysts obtained were higher with the addition of vitamin E only, than in media supplemented with both vitamin E and C (181, 182) On the other hand Wang et al found the addition of vitamin C to culture media more effective than vitamin E in reversing ROS induced mouse embryo toxicity (183). Supplementation with taurine and hypotaurine improved blastocyst development and hatching and reduced embryo apoptosis (7). The role of growth factors such as insulin growth factor (IGF)-I, IGF-II and epidermal factor (EGF) were evaluated in respect to mouse embryo dynamics including inner cell mass in developing blastocyst. Kurzawa et al tested the protective activity of these growth factors against hydrogen peroxide induced OS in mouse embryos. IGF-I, IGF-II and EGF increased the total blastocyst count, as well as the inner cell mass in developing blastocyst .Similar benefits of IGF-I and its de phosphorylated binding protein (IGFBP) were seen in terms of blastocyst formation and total cell number of blastocyst, when added to culture medium of developing mouse embryos (184).The beneficial influence of growth factors on embryo development in unfavorable milieu has not been well explained . Perhaps these growth

factors act by inhibiting the process of apoptosis. These factors had a positive effect on preimplantation embryos compensating for the conditions of OS (185).

Besides supplementation, modifying the embryo environment by eliminating ROS producing sources such as light and high oxygen concentrations can improve culture conditions. Oxygen concentrations in the oviduct are one quarter to one third the atmospheric tension. Modification such as utilizing lower oxygen tension (5%) has shown benefit in human embryos and resultant improved implantation rates (186). Reduced exposure to light or manipulating embryos in actinic light has been shown to be beneficial (7). Even though the presence of OS is inevitable in culture media, understanding the metabolism of gametes and preimplantation embryos may further improve culture conditions in near future.

OS seems to be involved in a plethora of processes of female reproduction, with definite role in varied aspects of assisted conception. 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

Conclusion

A review of the existing literature demonstrates the role OS plays in modulating a gamut of physiological functions and its role in pathological processes affecting the female reproduction. OS modulates a host of reproductive pathologies affecting natural fertility in a woman's life and also menopausal transition and post menopausal years. The role of OS is becoming increasingly important as there is new cumulative evidence suggesting that oxidative stress is involved in conditions such as abortions, preeclampsia, hydatidiform mole, fetal teratogenecicity, preterm labor and intrauterine growth retardation, all of which lead to an immense burden of maternal and fetal, morbidity and mortality. Explicating the role of OS in unexplained infertility and recurrent pregnancy loss is important to design strategies to ameliorate the OS related adverse effects. Preeclampsia is a disease with a huge magnitude of disease burden, affecting 10% of first time pregnancies. Oxidative stress has been found to play a pivotal role in its pathogenesis. OS seems to be involved in adverse affect on natural fertility, with definite role in varied aspects of assisted conception. Optimizing various techniques in the ART laboratory can be an effective strategy to intercept OS in IVF/ICSI and IVM settings. There is ongoing debate on the role of antioxidants in modifying the disease outcomes. The outcomes of the studies reviewed need validation by larger randomized, double blind and case-controlled trials.

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