Antioxidants and female reproductive pathologies

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Submitted: 14 October 2008
Accepted: 28 November 2008

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A b s t r a c t

Oxidative stress, the imbalance between pro-oxidants and antioxidants, has been evidenced in a number of female reproductive pathologies, and studies suggest its function in the etiology of many of these diseases. This review investigates the role of oxidative stress specifically in pregnancy-associated complications as well as infertility-associated complications. Placental oxidative stress has been implicated in the pathogenesis of antiphospholipid syndrome and hyperhomocysteinemia, which comprise the etiology of recurrent pregnancy loss (RPL). Oxidative stress also has been documented in relation to idiopathic RPL cases, hydatidiform mole, and preeclampsia. Oxidative stress can induce DNA damage and apoptosis, thought to be responsible for the pathophysiology of hydatidiform mole. Additionally, macrophage activation due to retrograde menstruation can induce oxidative stress, ultimately leading to endometriosis, while stimulating its proliferation. A decrease in antioxidants along with placental oxidative stress in pregnant women may activate uncontrolled lipid peroxidation, causing vascular endothelial damage resulting in preeclampsia. For women with hydrosalpinx, the presence of oxidative stress in the hydrosalpingal fluid may be the root cause of embryotoxicity. A persistent increased generation of reactive oxygen species in the peritoneal cavity seems to provide an explanation of unexplained infertility. Finally, increased oxidative stress also seems to be responsible for diminishing oocyte quality, thereby leading to oocyte aging. However, it is important to note that no widespread consensus has been reached. Continued investigation is essential because if oxidative stress is, in fact, an important part of the pathophysiology of these diseases, antioxidant supplementation may serve to reduce symptoms, restore normal function, or prevent the development of these diseases in at-risk women.

K e y w o r d s: recurrent pregnancy loss, preeclampsia, hydatidiform mole, endometriosis, hydrosalpinx, unexplained infertility, premature ovarian aging.

I n t r o d u c t i o n

A wide array of female reproductive diseases exists, each with a different etiopathogenesis. A common underlying factor identified in a number of these pathologies is oxidative stress (OS). As discussing all female pathologies is beyond the scope of this review article, we turn our attention to the more commonly occurring pathologies in which OS has been heavily implicated. These specifically include pregnancy-associated complications, particularly recurrent pregnancy loss (RPL), preeclampsia, and hydatidiform mole. Also included are infertility-associated pathologies encompassing endometriosis, hydrosalpinx, unexplained infertility, and premature ovarian aging.
In the normal physiological capacity, a delicate balance exists at the molecular level between oxidants and antioxidants. Oxidants are broadly categorized as reactive oxygen species (ROS) and reactive nitrogen species (RNS). The common ROS include, but are not limited to, superoxide ion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl ions (OH$^-$. Major RNS encompass nitric oxide (NO), nitrogen dioxide and peroxynitrite. To counterbalance the oxidant capacity and provide a protective means from the potential toxic effects of these oxidants, the function of antioxidants (AOs) is introduced. Antioxidants can be defined as any substance that when present at concentrations lower than an oxidizable substrate significantly delays or prevents oxidation of that substrate [1]. Antioxidants are present in enzymatic and non-enzymatic forms. Thus, in the presence of a disturbed balance between oxidants and AOs, OS ensues.

Over many years, researchers have demonstrated the role of OS in many disease processes affecting almost every organ system of the human body. This led researchers to believe that OS has a role in the female reproductive system and subsequent pathologies. With this review article, our goal is to define the exact role of OS in the evolution of the aforementioned disease processes in the female reproductive system in an attempt to direct efforts at overcoming the deleterious impact of OS.

**Pregnancy associated complications**

In this section we explore the relation between the common pregnancy-associated complications and OS.

**Recurrent pregnancy loss**

Recurrent pregnancy loss is defined as three or more consecutive pregnancy losses before 20 weeks gestation. This is a relatively common phenomenon seen in 0.5 to 3% of women in the reproductive age group. It is a frustrating problem for both clinicians and patients. Along with its clinical implications, RPL has an immense psychological effect on the patient and her family. The loss of a pregnancy can have devastating effects for a couple, regardless of family size or the cause for pregnancy loss.

Any discussion of RPL cannot exclude mention of spontaneous abortion. Although defined as two separate entities, repetitive spontaneous abortion relates to RPL in the mere fact that it is early pregnancy loss and, in more exact terms, defined as the termination of pregnancy before the age of fetal viability, i.e., 20 completed weeks of gestation or fetal weight less than 500 g (World Health Organization). The rate of single spontaneous abortion in North America is 15 to 20% among clinically diagnosed pregnancies and may be up to 31% of the total number of pregnancies [2]. Incidence increases with age; from 15% at 25 years of age to 35% in women older than 38 years [3]. The incidence not only varies with age but also varies with the number of previous spontaneous abortions. Women with a past history of one miscarriage have a 23% chance of another miscarriage in their next pregnancy. Likewise, two miscarriages increase the risk to 29% in the following pregnancy, and four miscarriages increase the risk to 33% in subsequent pregnancy, thereby falling into the definition of recurrent pregnancy loss. Recurrent pregnancy loss requires close evaluation because women with a history of RPL are at increased risk of suffering an early pregnancy loss in their subsequent pregnancy as compared with the general population.

**Etiopathogenesis of recurrent pregnancy loss**

Myriad causative factors have been identified in the development of RPL. These include chromosomal and genetic anomalies, uterine anomalies, blood abnormalities and autoimmune diseases. Similarly, studies have identified increasing maternal age, molar pregnancy, hydrosalpinx, and polycystic ovarian syndrome (PCOS) as some of the causative factors leading to spontaneous abortions and possibly contributing to the etiopathogenesis of RPL. However, even with the numerous identified causative factors, only a small percentage of RPL cases have distinct etiologies; the remaining 50 to 60% of cases still present with the clinical challenge of an ambiguous etiology. The factors thought to be related to the underlying pathophysiology of idiopathic RPL include endothelial damage, impaired placental vascularization, and immune impairment. Moreover, recent studies have begun to explore the role of OS in RPL.

Various literatures have documented increased OS during pregnancy. This has been demonstrated by numerous studies assessing a variety of oxidative stress markers identified in pregnancy. Early pregnancy is accompanied by a rise in polymorphonuclear (PMN) leukocytes. Studies suggest a relationship between increased PMN leukocyte levels and the associated increased production of O$_2^-$ free radicals [4]. An even higher production of ROS has been documented in recurrent abortion patients [5]. Additionally, elevated plasma levels of lipid peroxides and glutathione, as well as lower levels of antioxidants, such as vitamin E and β-carotene, have been demonstrated in RPL patients [6]. Miller et al. [7] further substantiated this finding by a study in which they observed a significant increase in plasma glutathione levels in RPL patients. Similarly, a decreased concentration of plasma...
ascorbic acid, α-tocopherol, total thiols, and erythrocyte-reduced glutathione (GSH) was seen in patients with unexplained RPL as well as patients with autoimmune and luteal phase insufficiency. The findings of these various studies reflect an increase in oxidative stress in these patients [8]. Conversely, Nicotra et al. [9] demonstrated no significant difference in plasma levels of triglycerides, cholesterol, cholesterol esters, phospholipids, liperoxides, vitamin E, or erythrocyte glutathione peroxidase activity in women with recurrent abortions as compared with controls.

**Placental oxidative stress**

The human placenta is hemochorial, in which the membrane enclosing the fetus comes in direct contact with the maternal blood. Adequate uteroplacental circulation is one of the prime requirements for a successful pregnancy. Initially, the tips of the spiral arteries are occluded by plugs of endovascular trophoblast cells arising from the trophoblastic shell [10]. Dislocation of these plugs due to invasion of trophoblasts allows circulation of maternal erythrocytes in the intervillous space, with a subsequent three-fold rise in oxygen tension between weeks 10 and 12 [11, 12], thus establishing the uteroplacental circulation [13-15]. The rise in oxygen tension is associated with a burst of placental oxidative stress as a result of the reperfusion of the ischemic tissue [16].

Abnormal placentation appears to be involved in the pathophysiology of RPL [17, 18]. In observing the trophoblastic shell in early pregnancy failure, approximately two-thirds of the cases present with thinning, fragmentation, and reduced endovascular invasion of the tips of the spiral arteries. In the case of a miscarriage, early dislodgement of trophoblast plug occurs, leading to precocious and generalized placental circulation and subsequent OS. This has been demonstrated *in vivo* by Doppler ultrasound imaging [19-22]. During this period, syncytiotrophoblasts do not have adequate antioxidant defense mechanisms and are subjected to oxidative damage [23]. Although *in vitro* studies demonstrate a regenerative capacity in syncytiotrophoblasts [24], with extensive damage incurred by OS, this capacity is overwhelmed and leads to pregnancy failure. However, in the event of a less severe insult, syncytial regeneration ensues and maintains the hormonal concentration required for the pregnancy, thus allowing the pregnancy to continue as a missed abortion [25].

In a case-control study, conducted by Jauniaux et al. [25], placental circulation was studied by Doppler ultrasonography in 65 pairs of age-matched normal and abnormal pregnancies. In normal pregnancies, intervillous blood flow increased with gestational age, being detected in 9 of 25 cases at 8 to 9 weeks and in 18 of 20 cases at 12 to 13 weeks. In abnormal pregnancies, flow was detected in nearly all cases (22 of 25) at 8 to 9 weeks. Early flow was restricted to the peripheral regions of most normal placentas, whereas in missed miscarriages it was most common in central regions or throughout the placenta. From this study the authors conclude that oxidative damage to the syncytiotrophoblast, induced by premature and widespread onset of the maternal placental circulation secondary to shallow trophoblast invasion, is a key factor in early pregnancy loss. High oxygen concentrations in the periphery of normal early placentas similarly may induce local regression of the villi, leading to formation of the chorion leve [17].

From these studies the role of oxidative stress behind the etiopathogenesis of recurrent pregnancy loss due to abnormal placentation has been defined.

**Antiphospholipid syndrome**

Antiphospholipid syndrome (APS) is a disorder of coagulation that causes thrombosis in both arteries and veins and has been identified as a risk factor in RPL. It presents as an autoimmune syndrome in which auto-antibodies are produced against phospholipids. It is commonly seen in conjunction with other autoimmune diseases, such as systemic lupus erythematosus (SLE). Antiphospholipid antibodies (aPL-Abs) are present in 30 to 40% of SLE patients and contribute to a significant proportion of RPL in this group. Primary APS is defined as APS occurring in the absence of any other related autoimmune disorder. Primary APS has been documented as contributing to 10 to 15% of RPL cases [26, 27]. Carvera et al. (2008) [28] conducted a multi-center prospective trial with the goal of evaluating the APS-related morbidity and mortality in 1000 patients during a five-year period. The findings of the study showed a total of 77 (9.4% of patients) women experiencing one or more pregnancies and 63 women (81.8% of pregnant women) successfully having one or more live births. The findings further observed the common fetal complications associated with APS patients. These included early pregnancy loss (17.1% of pregnancies), late pregnancy loss (6.7% of pregnancies), premature birth (35% of live births) and intrauterine growth restriction (13.7% of live births). Furthermore, the study demonstrated maternal morbidity as a relatively common occurrence in patients with APS. These included cases of preeclampsia, eclampsia and abruptio placentae.

Abnormal placentation as a result of impaired trophoblast invasion has been suggested to play an important role in aPL-Ab-associated RPL [29].
Further supporting this notion are studies demonstrating the direct effect of aPL-Abs on placental components, maternal decidua, and invading trophoblasts. aPL-Abs act by binding to the trophoblast monolayer and inducing (i) a direct cellular injury; (ii) apoptosis; (iii) inhibition of proliferation and syncytiotrophoblastic formation; (iv) decreased human chorionic gonadotrophin (hCG) production; and (v) defective invasiveness [30-33]. More recent research demonstrates effects of aPL-Abs on the maternal portion of the placenta. More so, normal implantation is further compromised in the presence of impaired endometrial differentiation and lower expression of complement regulatory proteins (DAF/CD55). Studies suggest these factors predispose to complement-mediated pregnancy failure [34, 35]. Various researchers have also demonstrated aPL-Ab-induced apoptosis further negatively compounds placentaion [36, 37].

Studies document that inflammatory processes occurring at the placental level can be mediated by aPL. It has been indicated in animal models that aPL-mediated inflammatory processes account for fetal loss [38-41].

An important role for OS has been documented in the pathophysiology of APS. Extensive peroxidation of the low density lipoprotein (LDL) leads to changes in the antigentic characteristics of other oxidized phospholipids. This makes them susceptible to the aPL-Ab [42]. Ox-LDL, by interacting with β2GPI via LDL-derived ligands, forms ox-LDL/β2GPI complexes [43]. These complexes have been found in patients with SLE and APS [44, 45].

Redecha et al. (2007) [46] conducted an animal study demonstrating that binding of C5a to its receptor induces the pro-coagulant molecule tissue factor (TF) expression in neutrophils, eventually contributing to respiratory burst and subsequent trophoblast injury and pregnancy loss induced by aPL antibodies. The study concluded that TF is an important mediator of C5a-induced oxidative burst in neutrophils in aPL-induced fetal injury. They suggested that complement activation is initiated by aPL-IgG binding to trophoblasts which leads to generation of the anaphylotoxin C5a; C5a then attracts and activates neutrophils. Resultant neutrophil TF expression enhances neutrophil oxidative burst leading to decidual injury and fetal death.

Low-dose aspirin along with low molecular weight heparin can increase the pregnancy rate in the patients with APS and other autoimmune thrombophilias [47].

**Effect of oxidative stress on spermatozoa**

Oxidative stress leads to sperm DNA damage during sperm transport through the seminiferous tubules and epididymis [48-51]. This may result in primary damage such as single-stranded and double-stranded DNA fragmentation and secondary damage such as generation of B-OH-2'-deoxyguanosine, a defined marker of DNA damage. Fertilization of the oocyte by a spermatozoon with damaged DNA has been documented to play a role in implantation failure, embryo developmental arrest, pregnancy loss or birth defects [48, 52, 53]. Recent studies have further demonstrated that sperm DNA fragmentation may be associated with an increase in sperm aneuploidy [54]. It is suggested that aneuploid sperms are more susceptible to the deleterious effects of oxidative stress, namely DNA fragmentation, during their passage through the epididymis [55]. Thus, sperm DNA fragmentation testing should be considered in couples diagnosed with RPL.

**Hyperhomocysteinemia**

Homocysteine, a thiol-containing amino acid, is proposed to have pro-oxidant effects. Plasma homocysteine levels normally decrease during pregnancy. Hyperhomocysteinemia has been associated with fetal neural tube defect, RPL, preeclampsia, and placental abruption [56-58]. Homocysteine also leads to abnormal placentation by damaging both the decidual and the chorial vessels [59, 60]. A meta-analysis by Nelen et al. (2000) [59] concludes that hyperhomocysteinemia (a fasting total homocysteine level >18 mmol/l) is a risk factor for RPL. This is in support of the study conducted by Wouters et al. (1993) [61] and Steegers-Theunissen et al. (1992) [58]. Del Bianco (2004) [62] also showed that hyperhomocysteinemia may be a cause of RPL. *In vitro* studies suggest that homocysteine is related to endothelial dysfunction, smooth muscle cell proliferation, and abnormalities of coagulation responsible for the pathogenesis of vascular disease. In addition, homocysteine generates ROS such as H₂O₂ that may induce OS and thereby endothelial dysfunction. Timely identification of hyperhomocysteinemia in women with RPL will give us a chance to normalize the homocysteine levels and may help us to achieve normal births [63].

The vitamins folate and cobalamin are involved in the remethylation of homocysteine into methionine, and vitamin B₁₂ (pyridoxal 5-phosphate) is a cofactor in the transsulfuration of homocysteine by causing conversion of cystathionine to cysteine. Hyperhomocystinaemia results either due to a hereditary defect within the methionine-homocysteine pathway, or it might be acquired as a result of deficiencies of vitamin B₁₂, folate (B₉) [60] and vitamin B₆ or due to genetic factors such as mutation in the methylene tetrahydrofolate reductase (MTHFR) gene which results in decrease in the activity of the enzyme and increase
the homocysteine concentrations in blood [64, 65]. The homocysteine level also rises with age, smoking, and coffee intake and also varies with gender (higher in men than in women) [66].

Interestingly, a study conducted by Sutterlin (1997) [67] failed to show any correlation between the levels of folate, vitamin B$_{12}$ and the number of previous abortions. The main drawback of this study was the small sample size used—29 nonpregnant women with a history of RPL of unknown etiology were compared to 29 healthy nulligravidae of similar reproductive age (controls).

A positive association between number of failed pregnancies and dysfunctional folate metabolism has been demonstrated [59, 65, 67]. These deficient folate levels may be a reason behind hyperhomocysteinemia. Obstetricians have advocated folic acid supplementation to normalize the levels of homocysteine. Quéré et al. (2001) [68] were able to achieve a pregnancy rate of 20/25 women by administration of folic acid.

In a follow-up study conducted by Bennett et al. (2001) [69], vitamin B$_{12}$ supplementation allowed successful pregnancies in all except one case out of 15 women with RPL, who had a combined heterozygous factor V mutation. They emphasized the implication of vitamin B$_{12}$ deficiency in RPL and infertility.

In a case report by Candito (2003) [70], hyperhomocysteinemia and low vitamin B$_{12}$ levels were found in a 38-year-old woman with a history of four RPL. Parenteral B$_{12}$ therapy was successful in normalizing the homocysteine levels in 2 months and achieving pregnancy.

Recently Nadir et al. (2007) [71] studied the association between homocysteine, vitamin B$_{12}$, folic acid, and MTHFR C677T as the levels of homocysteine, vitamin B$_{12}$, and folic acid and the MTHFR C677T genotype are linked biochemically. They found a weak association between homocysteine and related parameters. Highly elevated homocysteine, observed in the minority of patients, was the only case in which a positive correlation with the levels of vitamin B$_{12}$ and folic acid was found. The results were in association with the studies that found no correlation between the vitamin B$_{12}$ and homocysteine levels [72-75], folate and homocysteine levels [72, 76], and MTHFR and homocysteine levels [77, 78].

Although folate, vitamin B$_{9}$, and B$_{12}$ supplementation has been shown to improve the pregnancy rate in the patients with hyperhomocysteinemia [47], no treatment guideline exists for patients with thrombotic events and normal homocysteine levels. Oger et al. (2006) [79] has reported that low serum folic acid as well as low vitamin B$_{12}$ levels were independently associated with venous thrombosis. Whether or not vitamin supplementation should be given to this patient group remains a clinical dilemma, and studies are required to investigate the long-lasting protective effect of vitamin B$_{12}$ and folic acid in these patients.

**Selenium deficiency**

Studies have been conducted in an attempt to establish a relationship between selenium levels and RPL. Selenium functions as a cofactor for the antioxidant enzyme glutathione peroxidase. Studies performed by Al-Kunani et al. (2001) [80] and Nicoll et al. (1999) [81] have failed to demonstrate any significant difference in selenium levels between patients and controls. On the other hand, Kocak et al. (1999) [82] and Kumar et al. (2002) [83] reported a significantly lower levels of selenium in the recurrent abortion groups. Further studies are warranted in this area to determine selenium’s role in recurrent pregnancy loss.

**Management of recurrent pregnancy loss: antioxidant supplementation**

The use of antioxidant supplementation in RPL patients has not yet been justified in spite of the fact that many researchers have documented a decrease in the levels of antioxidants in these patients. The lack of data from sufficient randomized controlled trials prevents this from being a potential management option. Studies have shown the beneficial effects of antioxidant supplementation in in vitro media [84-86]; however, human studies are lacking in definitive evidence. Crha et al. [87] observed higher vitamin C levels in follicular fluid of patients supplemented with vitamin C in comparison to controls. It was further observed that pregnancy rates were higher in the supplemented group than in the control group; however, the data did not prove to be statistically significant.

A recent meta-analysis was conducted by Rumbold et al. [88] to determine the effectiveness and safety of any vitamin supplementation on the risk of spontaneous miscarriage, maternal adverse outcomes, and fetal and infant adverse outcomes. It included 17 randomized and quasi-randomized trials that were conducted prior to conception, peri-conceptionally, or in early pregnancy (less than 20 weeks of gestation) to compare one or more vitamins with placebo, other vitamins, or other interventions. Tests for heterogeneity in the studies and causes of such heterogeneity were also determined. The vitamins that were supplemented included vitamin A alone or with folic acid, zinc or multivitamins; vitamin C with or without multivitamins or vitamin E; folate with or without multivitamins and/or iron, and multivitamins alone. Results reported miscarriages.
or stillbirths in 15 trials. Reporting for miscarriages was erratic due to differences in defining criteria. As for the primary outcomes of total fetal loss, early or late, no difference was seen between women given any type of vitamin compared with controls. Although the trials for multivitamins demonstrated a lower rate of total fetal loss for women given multivitamins with or without vitamin A, the authors did not find any strong correlation between vitamin supplementation and reduction in incidence of early or late miscarriage in other trials. Overall, the study concluded that vitamin supplementations may not play a significant role in the prevention of miscarriage.

Even though this study rules out the beneficial effects of vitamins, many studies demonstrate the increased rate of abortions due to lack of antioxidant defenses as discussed earlier. The significant evidence that OS plays an important role in the pathogenesis of RPL cannot be neglected. More specific studies to establish the safety and effectiveness of the use of antioxidants and vitamins are required in these patients.

**Preeclampsia**

Preeclampsia is a disorder that complicates pregnancy; it is characterized by hypertension and proteinuria beginning around the twentieth week of gestation [89-93]. Aside from affecting roughly 5 to 10% of pregnancies, it also is one of the leading causes of maternal and perinatal morbidity and death [89, 92, 94-99]. Delivery is recognized as the most effective form of treatment, oftentimes resulting in premature birth [92, 93, 100, 101]. First-time mothers are considered to be at a greater risk [102]. Other common characteristics of preeclampsia include: generalized vasoconstriction, increased vasoactivity, reduced perfusion to organs, and platelet activation [90]. The exact causes and mechanisms of this disorder are not fully understood. It is believed, however, that maternal vascular endothelium dysfunction is a factor that triggers its onset [89, 97, 98, 103, 104]. Many hypothesize that OS is a key factor in this endothelial dysfunction.

As mentioned earlier, pregnancy is accompanied by the placional increase of ROS and RNS levels in the mother; however, the pregnancy will progress without complications because her plasma activity levels of SOD increase as well in order to buffer the ROS, preventing OS [18, 92, 105, 106]. Lipid peroxidation, the oxidative degradation of lipids by free radicals, however is uncontrolled in preeclampsia causing vascular endothelial damage, ultimately resulting in the hypertension and proteinuria that characterize the disorder. Many believe that the etiology of preeclampsia lies in depletion of antioxidants [92]. In these women, it has often been found that there is a reduction in antioxidants such as SOD in both the placenta and maternal blood leading to OS [107-110]. Reduced levels of the antioxidants SOD and glutathione peroxidase prevent NO from regulating blood pressure. With low levels of SOD, NO reacts with O$_2^-$ to form peroxynitrite, increasing OS [92]. It has been proposed that the decrease in activity of SOD is the result of a change in transcriptional regulation of SOD expression; however, Perkins et al. did not find a difference in SOD mRNA expression [105, 111, 112].

The theory of pro-oxidant and antioxidant imbalance suggests greater membrane lipid peroxidation consequently causing vascular endothelial damage [112, 113]. Lipid peroxidation occurs when oxygen free radicals interact with the polyunsaturated fatty acids in membranes and lipoproteins, resulting in OS. Since the accretion of maternal, placental and fetal tissue during pregnancy requires women to ingest more fatty acids, these diets might contribute to both the vasoconstriction and OS associated with preeclampsia [114-116]. Mehendale et al. found in a study of 60 preeclamptic women that MDA levels were significantly higher, a marker of OS and an end product of lipid peroxidation. Also, vitamin C and E levels were significantly lower. This suggests that women with preeclampsia have increased OS and reduced antioxidant defense. This imbalance leads to free radical-mediated cell injury and endothelial dysfunction. Additionally, increased lipid peroxidation would decompose polyunsaturated fatty acids, promoting inflammatory responses and vasoconstriction, which are observed in these women as well [91].

Dordevic et al. [90] came to similar conclusions. They evaluated 42 women, 20 of whom exhibited mild preeclampsia, to see if erythrocytes were damaged by OS and if that damage was related to the disease. Women with preeclampsia have significantly higher levels of leukocytes, which, when activated, release large amounts of ROS and RNS, capable of permeating the erythrocyte membrane and inducing OS [117-119]. This also was confirmed because they found higher concentrations of H$_2$O$_2$, NO$_2^-$, ONOO$^-$, and GST levels-markers of OS in the erythrocytes of women with preeclampsia. These pro-oxidant levels had significant positive correlation to leukocyte levels. Peroxynitrite is a source of OH$^-$ which oxidizes polyunsaturated fatty acids in membranes, thus inducing lipid peroxidation. The results of the study substantiate this because they found higher concentration of lipid peroxidation in the erythrocytes of these women [120]. Lipid peroxidation of the plasma membrane of erythrocytes reduces its hydrophobic characteristics thereby altering its affinity and interaction between proteins and lipids.
Consequently, erythrocytes are incapable of functioning normally [121].

While non-enzymatic components of the erythrocyte antioxidant defense did not differ between the controls and women with preeclampsia, the concentration of SOD, CAT, and glutathione reductase (GR) were significantly lower. The decrease in GR indicates that women with preeclampsia fail to maintain GSH concentrations as well.

Dordevic et al. ultimately found a significant positive correlation between mean arterial pressure, markers of OS, and oxidative damage in erythrocytes, suggesting that OS in erythrocytes is linked to endothelial cell dysfunction [90].

A study by Ryu et al. [93] also maintains the idea that OS leads to endothelial cell dysfunction, hence, resulting in preeclampsia. They found that leukocytes adhered more readily to endothelial cells cultured in the presence of plasma from women with severe preeclampsia than from plasma cultured from women with normal pregnancies. Additionally, vitamins C and E and N-acetylcysteine inhibited this adhesion, with vitamin E resulting in 66% less adhesion and similar results for vitamin C.

The role that antioxidants could play in the prevention and alleviation of preeclampsia is under much debate because there is a great deal of conflicting data concerning their effectiveness. One study found that vitamin C supplementation might be beneficial, while another found modest benefits [102, 124]. Others have found no significant value and no reduction in risk for preeclampsia [102, 125, 126]. Oxidative stress also is known to cause complications in preterm infants such as respiratory distress, chronic lung disease, and prematurity [127, 128]. Thus, many believe that antioxidant supplementation can both alleviate maternal preeclampsia and prevent perinatal complications.

In a large study of 739 patients conducted by Spinnato et al. [129], the use of vitamin C and E supplementation had no significant effect on the rate of preeclampsia. However, the study did not identify any deleterious effects of supplementation on low birth weight, small for gestational age, still birth, or birth asphyxia, some of which were found in the study conducted by Poston et al. Interestingly, this study also found a greater frequency of pre-mature membrane rupture. Other studies also have found no significant benefit of antioxidant supplementation [102, 125, 126, 130]. One of the studies also suggested that the risk of preterm births may actually be increased with vitamin C supplementation during pregnancy [131]. In the meta-analysis conducted by Rumbold et al., vitamin C and E supplementation did not reduce the risk of preeclampsia in women nor did it reduce the risk of serious outcomes in infants, although it did reduce the risk of respiratory distress syndrome. In fact, women taking supplementation were more likely to be treated for hypertension antenatally. This meta-analysis, however, cannot be generalized to women with low dietary intakes of antioxidants [102].

In contradiction to the other, a randomized, double-blind, placebo-controlled trial studied the effects of antioxidant supplementation during pregnancy for women with low antioxidant levels during early gestation. Overall, the supplementation group exhibited a significantly lower rate of preeclampsia, 2 cases vs. 9 cases. The subjects were given a very broad range of antioxidants, suggesting that a large variety of antioxidants might be required to protect from OS. This study demonstrated that antioxidants given earlier in gestation, during trophoblast invasion, may have better effects. Rumiris et al. [92] believed that in past studies, supplementation was administered too late to improve placental formation. This study, however, was limited by its small sample size. More evidence of antioxidant safety is needed as well before they are used as a preventative therapy.

Hydatidiform mole

Hydatidiform mole (H. mole) is a known placental malformation causing early miscarriage and accounts for 1 per 41 cases of early pregnancy loss (EPL) [132]. The complete or classical hydatidiform mole is defined as a conceptus with a placenta showing generalized swelling of the villi and diffuse trophoblastic hyperplasia, in the absence of an ascertainable fetus. The partial H. mole is characterized by focal trophoblastic hyperplasia with focal villous hydrops and identifiable embryonic or fetal tissue. The estimated incidence of partial mole is 1 per 700 pregnancies, whereas the incidence of complete mole is around 1 per 1500-2000 pregnancies [132, 133]. Apart from causing early pregnancy loss, H. mole needs to be further studied because of the well-established risk of persisting gestational trophoblastic disease and or development of choriocarcinoma after surgical evacuation or delivery.

From time to time various studies have pointed towards the role of OS in H. mole. However, whether OS play a causative, incidental, or secondary role is not clear.
Harma et al. (2003) [134] studied the oxidative status and antioxidant status of plasma of 38 patients with complete H. mole (CHM) (mean gestational age 12.9 weeks as estimated by last menstrual period) and 31 healthy pregnant women (mean gestational age 13.2 weeks as estimated by ultrasonography). Obese women, underweight women, and smokers were excluded from the study. Total antioxidant potential (TAOP) was taken as the antioxidant status and plasma total peroxide levels were taken as the measure of oxidative status. The ratio of TAOP to total peroxide was accepted as an indicator of OS. They showed that plasma TAOP was significantly lower in patients with H. mole than in healthy pregnant women, and the mean OS index level was significantly higher in patients with CHM than in healthy pregnant women. The authors concluded that patients with CHM are exposed to increased OS, which may play a role in the pathogenesis of the disease or may be secondary to the disease.

In a similar study the protein carbonyl and total plasma thiol concentrations in patients with CHM were compared with those in healthy pregnant women [135]. Carbonyl derivatives of proteins, or protein carbonyls, may be sensitive biomarkers of ROS [136]. Plasma thiols are physiological free radical scavengers that serve as antioxidants by several mechanisms [137]. The study involved 80 women. Of these, 41 were healthy pregnant women (controls) in the first trimester of pregnancy with a single viable fetus (mean gestational age 13.0 weeks as estimated by ultrasonography). The remaining 39 patients had CHM (mean gestational age 14.2 weeks as estimated by last menstrual period). Obese, diabetic, vitamin, or other drug users, underweight women and smokers were excluded from the study. High levels of protein carbonyl content and slightly lower levels of plasma thiol concentrations were found in CHM patients. The authors suggested that elevated OS or disturbances in detoxification processes may contribute to the development of CHM.

Besides ROS, the role of NO in the CHM patients has been defined. A significant increase in concentration of NO was found in patients with CHM, and this was significantly associated with increased risk of CHM [138]. This raised NO levels have been attributed to the increased expression of endothelial NO synthase (eNOS) on the syncytiotrophoblasts [138].

Possible sources of oxidative stress in Hydatidiform mole

Altered placentation has been thought to be responsible for the generation of increased OS in these patients similar to preeclampsia [139]. In first trimester CHM the extravillous trophoblast invasion into the decidua and superficial myometrium is extremely shallow [31, 140]. In the implantation site of H. moles, trophoblastic atypia is diffuse [141]. There is a profound alteration in the biological function of the extravillous trophoblast due to defective expression of the metalloproteinases like osteopontin (OPN) [142], which leads to destruction of vessel walls and excessive entry of the maternal blood into the intervillous space. This results in direct mechanical damage of the vilous tissue and an indirect OS effect [143]. An increase in the number of infiltrating inflammatory cells in the molar implantation site [144] also suggests an abnormal maternal endometrial tissue response.

Harma et al. [135] in their study suggested that the observed elevation in protein carbonyls in CHM is largely due to the hypertrophied state of the placenta in CHM.

Besides placental OS, these patients also demonstrate inflammatory states similar to that seen in preeclampsia with increased levels of cytokines, including TNF-\(\alpha\), IL-1 and IL-6. Increased expression of IL-1\(\beta\) is associated with persistence of the disease and invasion in CHM [145]. Marked elevations of serum IL-1\(\beta\), IL-6, and TNF-\(\alpha\) are associated with progressive (high-risk) GTD, whereas only slight elevation of IL-6 and normal levels of IL-1 and TNF-\(\alpha\) are seen in remitting cases of H. mole [146, 147]. These raised cytokines levels are responsible for the activation of inflammatory cells that leads to ROS generation as well as endothelial cell injury.

The role of carotene also has been identified. Studies have shown that deficiency of carotene, a vitamin A precursor, is associated with the increased risk of developing CHM [148]. A significant trend for decreasing risk for molar pregnancy with increasing consumption of carotene also has been shown [148]. In contrast to this, the risk for partial H. mole is not increased with vitamin A or carotene deficiency [149].

Possible mechanism of oxidative stress induced damage in Hydatidiform mole cases

It has been proposed that OS leads to increased DNA damage. Harma et al. [150] studied the relationship between antioxidant levels and levels of endogenous DNA damage and found an inverse correlation between the two. The authors suggested that there is a link between increased levels of OS and the increase in endogenous DNA damage seen in patients with CHM, as compared with those seen in normal pregnancy.

Another way in which oxidative stress may produce effects is through increasing apoptosis. Bcl-2 expression was found to be comparatively weak in cytotrophoblasts and syncytiotrophoblasts in normal placenta and, although confined to the
Infertility associated pathologies

A number of female reproductive pathologies are complicated by female infertility. In this section we direct our attention to some of the more common pathologies including endometriosis, hydrosalpinx, unexplained infertility, and premature ovarian aging.

Endometriosis

Endometriosis is a disease characterized by chronic inflammation and the implantation and growth of endometrial tissue outside the uterine cavity. Apart from affecting 10 to 20% of women in the reproductive age group [154], it has been identified as a major factor in infertility, accounting for roughly 21 to 44% of all infertility cases [155-157]. Approximately 80% of endometriosis cases are complicated by infertility. The pathogenesis of endometriosis and its etiology is not completely understood, but Sampson’s theory of retrograde menstruation is widely recognized as a distinct possibility. During the shedding of the endometrium, some endometrial fragments pass through the fallopian tubes, ending up in the peritoneal cavity. As a result, these fragments then implant on the surfaces within the peritoneal cavity, proliferating with each new menstrual cycle [158]. A woman’s risk for endometriosis is increased with increased frequency of periods, longer periods, or less parity [159]. Essentially this implies that an increase in exposure of the peritoneum to menstrual fluid in the peritoneal cavity, producing free radicals leading to the peroxidation of lipids [166-168]. These peroxidized lipids generate products such as malonaldehyde (MDA) after their decomposition, which can be recognized as foreign bodies, triggering an antigenic response that produces antibodies. This response may lead to oxidative damage to red blood cells, endometrial cells, and peritoneal cells, stimulating more mononuclear phagocytes, thus perpetuating a cycle of oxidative damage [166-169]. Supporting this idea, Shanti et al. found increased autoantibodies related to OS in women with endometriosis [170]. Oxidative stress also damages mesothelial cells, providing sites for the adhesion of ectopic endometrial cells [163]. The generation of ROS from mononuclear cells can also have a direct effect on reproductive steps such as reproductive cell viability, sperm motility, oocyte fecundity, and implantation. This may begin to explain the infertility associated with endometriosis, but it is still not completely understood [171-174].

Moderate OS was found to induce the proliferation of endometrial tissue, so its presence in these women could explain the etiology and sustention of the disease [100, 175-178]. Thus, there has been a great deal of research to investigate if OS does play a part. Many studies have found that these women do have increased levels of OS, ROS, or enzymes that produce ROS [170, 179-182]. Jackson et al. found increased levels of thiobarbituric acid reactive substances (TBARS), a measurement primarily for MDA, in the serum of women with endometriosis, and it was weakly associated with an increased risk for the disease. However, other markers of OS were not associated with endometriosis [166]. Others also have found no association between OS and endometriosis or the stages of the disease [183-186]. Many found the presence of OS in women with endometriosis but when compared to controls the level of OS was not significantly higher [187]. Overall, great inconsistency exists among the various studies on the association between OS and endometriosis. Jackson et al. noted that comparisons can be difficult because of the variability in eligibility...
hydroperoxides are products of the peroxidation process. These lipid peroxides are visible on ultrasonogram [197-199]. This led to the belief that the hydrosalpinx fluid (HSF) plays a key role in implantation failure. Various studies were conducted to know the exact mechanism by which HSF exerted its deleterious effects.

Five out of eight studies using a murine model described embryotoxicity at low concentrations of human HSF, and three studies demonstrated impaired development in undiluted HSF [200]. Only two human studies have shown a 50% reduction in the blastocyst developmental rate in undiluted fluid as compared with control medium or diluted HSF [201]. Interestingly, the study conducted by Granot et al. (1998) [202] failed to report any effect on pregnancy rates in such cases.

Hydrosalpinx

Hydrosalpinx is a blocked, dilated, and fluid-filled salpingeal tube usually caused by previous tubal infection. Many investigators have reported low pregnancy and implantation rates as well as higher rate of spontaneous abortions in patients with hydrosalpinx.

Proposed pathophysiology behind lower implantation rates

Embryotoxic effect of hydrosalpinx fluid

Removal of hydrosalpinx by salpingectomy has been shown to improve pregnancy rates, particularly in patients with large hydrosalpinges that are visible on ultrasonogram [197-199].
blastocyst development in undiluted HSF. Strandell et al. concluded that lack of essential substrates in HSF may be responsible for the harmful effects [203]. Chan et al. (2003) [204] evaluated the morphologic and growth parameters of embryos, i.e. crown-rump length, yolk sac diameter, and number of somites, in the study group embryos exposed to 20% HSF and compared it with control group embryos exposed to 20% lactated Ringer’s solution. This study showed that HSF has a significant toxicity on rat embryos during the period of organogenesis and has effects on the morphology as well. The results from these studies done on the animal model cannot be applied to humans. One of the reasons for this would be that the murine embryos were exposed to HSF from a different species.

Some substances in HSF may be responsible for its embryotoxicity. HSF may contain microorganisms and cellular debris that are toxic to embryos [205]. In agreement with this, Meyer et al. (1997) [206] came up with a proposition that an embryotoxin present in HSF may be responsible for the adverse outcomes associated with hydrosalpinx.

**Does oxidative stress have a role in the pathophysiology?**

Since the exact mechanism of embryo toxicity is not known, it can be hypothesized that OS may be an underlying factor. Bedaiwy et al. (2002) [186] in their study have described a positive effect of ROS in relation to blastocyst development, as compared with the absence of ROS in the HSF. In relation to this study, our group analyzed the effects of ROS, lipid peroxidation (LPO) products, and non-enzymatic total antioxidant capacity (TAC) levels in HSF of 11 hydrosalpinx patients on a two-cell mouse embryo culture system. The authors concluded that low levels of ROS (below the threshold for being deleterious to embryos) were significantly related to blastocyst development and may represent normal ROS generation by a functional endosalpinx, whereas HSF with non-detectable ROS levels may be derived from hydrosalpinges with more extensive endosalpingeal damage. Thus, the detection of low levels of ROS would indicate normal secretory functions. However, ROS also may be generated by the inflammatory cells of chronic salpingitis [207]. Low LPO levels were found in the HSF samples that had no significant effect on blastocyst development. This means that OS reaction occurs only during the acute phase of the disease. Low concentration of TAC was detected. It is unlikely that the extremely low concentrations of TAC would affect the blastocyst developmental rate directly.

A study conducted by Chanr et al. (2004) [208] is in disagreement with the hypothesis that OS has a role in the HSF-related toxicity. The authors studied the relationship between free oxygen radical levels and HSF-induced embryotoxic effects in rat embryos by measuring the embryonic 8-isoprostaglandin F2α level and the total protein contents in both embryos and yolk sacs. Measurement of 8-isoprostaglandin F2α level is a sensitive and specific method for the assessment of OS and damage, as shown by Morrow et al. (1991) [209]. The study demonstrated that free oxygen radical levels were not higher in embryos with morphological abnormalities. Therefore, authors concluded that HSF-induced embryotoxicity is not mediated through the excess production of free oxygen species and suggested that ROS detected in HSF may simply represent a normal by-product of cellular mechanism and were not responsible for the embryotoxic effects. Further studies are required to investigate the mechanism of HSF-induced embryotoxic effects.

**Effect of concentration of energy substrates required for the embryo development**

Studies on embryo development in HSF suggest a lack of necessary nutrients responsible for the impaired development of embryo to blastocyst. Energy requirements change from pyruvate to glucose during the development of the embryo to blastocyst. Low glucose concentration in the culture media has been suggested by Conaghan et al. (1993) [210]. Glucose concentration was found to be very low in HSF when compared with the levels in normal human tubal fluid and culture media [211, 212].

**Effect of hydrosalpinx fluid on integrins and cytokines levels**

It has been hypothesized that HSF may hinder implantation by reducing the endometrial integrins either due to their decreased expression [206] or due to blockage [213]. Integrins can be restored to normal with the removal of hydrosalpinx segment, thus improving implantation rates. Cytokines like IL-1, IL-1β, leukemia inhibitory factor (LIF), and colony stimulating factor (CSF-1) have a role in implantation. The presence of HSF has been proposed to cause defective secretion of all or some of these cytokines, leading to impaired embryo development and implantation [203]. More studies are required in this context. Higher concentration of TNF-α also has been found in these patients.

**Mechanical effect of hydrosalpinx fluid**

Another school of thought suggests that the flow of HSF from large hydrosalpinges into the endometrial cavity may mechanically flush embryos from the uterus [214-216]. Contradictory to this, a recent study has demonstrated an association...
between decreased implantation rate and endometrial cavity fluid in patients with hydrosalpinx that were not visible on sonogram [217]. A few researchers have suggested that the HSF may act as a physical barrier to implantation [99, 214-218]. However, this is not the sole reason for pregnancy failure in patients with hydrosalpinx.

Can hydrosalpinx-induced change in the endometrial peristalsis be a possible factor?

Altered endometrial peristalsis is seen in patients with hydrosalpinx. Normally, endometrial activity increases from menstruation to ovulation and decreases during the post-ovulatory phase. No waves from fundus to cervix occurred after ovulation in the pregnant group [219]. A switch in endometrial wave direction (WDS) from fundus-cervix to the direction cervix-fundus is described in *in vitro* fertilization (IVF) cycles [220]. In one recent study, the pressure gradient of the tubal fluid generated a reflux phenomenon opposing the cervix-to-fundus intrauterine peristalsis. This reflux phenomenon could be the reason behind the reduced implantation rates associated with hydrosalpinx [221].

Taking into account all the possible mechanisms involved in the pathogenesis of low pregnancy rates in hydrosalpinx, the low implantation rates in these patients may be a result of the mixture of the above mentioned factors and not due to a single factor.

Treatment options and the role of antioxidants in overcoming the effect of oxidative stress

Laparoscopic salpingectomy followed by IVF has been described as the only effective treatment for improving the pregnancy rates in these patients [197-199, 222-224]. Strandell et al. (2000) [200] advocated that reconstructive surgery should be used as a primary treatment instead of salpingectomy and IVF in hydrosalpinges with preserved mucosa. The latter may be useful as a secondary treatment option after failed conception and re-occlusion of the tubes. Unnecessary salpingectomies may easily be avoided by appropriate evaluation of the tubal mucosa by tubal endoscopy at laparoscopy before any final decision of salpingectomy is made. This practice can be very helpful in reducing the incidence of ectopic pregnancies as salpingectomy is associated with an increased risk of ectopic pregnancy post-surgery [225].

Later Strandell et al. (2002) [197] also concluded that patients with large hydrosalpinges and without the prospect of spontaneous conception should be recommended for salpingectomy prior to IVF as it certainly increases the chances of a successful IVF treatment.

Since OS has been believed to be a factor in the pathophysiology of hydrosalpinx, studies are required to investigate the role of antioxidant supplementation in these patients. Reactive oxygen species generation in a mouse IVF model has been shown to impede preimplantation embryo development and supplementation with antioxidants reverses this effect [226-228]. Studies in a human model to analyze the effectiveness of antioxidant supplementation in these patients are needed.

Unexplained infertility

Idiopathic (unexplained) infertility is a diagnosis of exclusion and is defined as the inability to conceive after 12 months of timed, unprotected intercourse where tests have been performed on both partners to rule out known causes of infertility, including but not limited to anovulation and sperm defects [229]. It affects 15% of couples [230]. The pathophysiology of unexplained infertility remains unclear and has been thought to be caused by increased generation of ROS in the peritoneal cavity. Wang et al. (1997) [185] found higher concentrations of ROS in women with idiopathic infertility in unprocessed and processed peritoneal fluid specimens, and these differences were statistically significant in the processed peritoneal fluid. Polak et al. (2000) [231] did a study to evaluate the activity of an extracellular superoxide dismutase (EC SOD) and TAC in peritoneal fluid (PF) and plasma from women with unexplained infertility. They showed that TAC was significantly lower in PF from women with unexplained infertility, and there was no significant difference in plasma TAS between the groups. They also found a positive correlation between PF and plasma TAC. Activity of EC SOD did not differ significantly between the groups in either PF or plasma samples. From this study they concluded that the low antioxidant status of PF but not that of blood may be responsible for idiopathic infertility, and the activity of EC SOD does not seem to play a role in unexplained infertility. The hypothesis that the low antioxidant status of the PF may be a causative factor for idiopathic infertility has been supported by a more recent study by the same group [161]. They speculated that PF diffuses into the fallopian tubes where it may cause damage to sperm, which are known to be sensitive to OS [232]. Concentrations of malondialdehyde, a lipid peroxidation end product, in peritoneal fluid were higher in patients with unexplained infertility than in fertile women [162, 233]. An increased peritoneal level of NO is seen in patients with idiopathic infertility [234]. Thus, it can be concluded that women with idiopathic infertility have reduced concentrations of antioxidants and increased ROS-induced lipid peroxidation damage, which results in infertility.

Studies have been done in the past to elucidate the role of antioxidants in the management of unexplained infertility. In one study, which dates
Antioxidants and female reproductive pathologies

back to 1994, women suffering from unexplained infertility, with significantly low levels of RBC glutathione peroxidase (GSH Px) were administered daily oral selenium as selenomethionine and oral magnesium supplements for 2 months, which normalized their RBC-Mg and RBC-GSH-Px levels. These women were able to achieve pregnancy, conceiving within 8 months of normalizing their RBC-Mg levels [235].

N-acetyl cysteine (NAC), by virtue of its ability to reduce extracellular cysteine to cysteine and being an excellent source of a sulfhydryl group, is known to be a powerful antioxidant. It also has antiapoptotic effects [50, 236], preserves vascular integrity, lowers serum homocysteine levels, and is protective against ischemic injuries [237-240]. N-acetyl cysteine has immunological functions also; it has an anticytokine effect and inflammatory-modulating capacity [241]. Conversely, Kleinfeld et al. [238] reported that NAC may act as a prooxidant in healthy individuals and may lower the glutathione and increase the amount of oxidized glutathione. N-acetyl cysteine has insulin-sensitizing capabilities and hence has been used successfully as an adjuvant to clomiphene citrate (CC) in PCOS patients to augment ovulation. However, its role in ovulation induction in patients with unexplained infertility is not clear. Recently Badawy et al. (2006) [242] conducted a randomized, double-blind trial to compare the effect of CC plus NAC vs. CC only for augmenting ovulation in the management of unexplained infertility. They showed that NAC was ineffective in inducing or augmenting ovulation in patients with unexplained infertility and suggested that NAC cannot be recommended as an adjuvant to CC in such patients.

Whether or not antioxidants will be helpful in patients with unexplained infertility is still a subject for debate. Attempts should be made to explore the management options in these patient groups since this affects a large population.

Premature ovarian aging

In women optimal fertility is maintained until 30 years of age and then decreases sharply with complete loss of fertility by the late forties. The change in the reproductive behavior of women as the consequence of increasing rate of education and employment has resulted in the postponement of child-bearing. Subsequently this has increased the mean female age into the late thirties for first childbirth [243, 244]. Increasing maternal age decreases the probability of achieving pregnancy within a period of one year; a condition referred to as subfertility. Clinicians and researchers have been showing an increasing interest in the reasons behind the decline of fertility with age among women, mainly due to the inability of assisted reproductive technologies (ART) in overcoming subfertility.

During fetal life, ovaries contain a huge store of ovarian follicles that decreases as the woman ages, with a marked increase in the rate of disappearance from age 37-38 years onwards. At menopause the number decreases to less than a thousand follicles, insufficient to sustain the cyclic hormonal process necessary for menstruation [245]. In addition to the decrease in the number of follicles with increasing age, a decline in the oocyte quality also has been proposed by many researchers. A study conducted by Sauer et al. (1990) [246] showed that oocytes donated from young to older women in assisted reproduction programs were able to overcome the effects of age on fertility, resulting in pregnancy rates similar to those observed in young patients. This provides direct evidence in support of oocyte aging. Poor oocyte quality is a major cause for the aging-related decline in female fertility [247].

Derangement of intracellular calcium homeostasis plays a major role in the deterioration of oocyte quality following maturation/ovulation [248-250]. Resultant secondary activation of cell cycle machinery causes a drop in the activity of the cell cycle factors, namely M phase promoting factor (MPF) and mitogen activated protein kinase (MAPK) [249, 250]. Both MPF and MAPK are important for the completion of meiosis and initiation of normal embryo development [251]. Premature advancement of cell cycle occurs before fertilization as a result of the drop in MPF and MAPK. Cortical granule (CG) loss, zona pellucida (ZP) hardening, and changes in spindle and ooplasmic microtubule dynamics, which are the characteristics of oocyte aging, also accompany this [250, 252-254].

It has been proposed that OS-induced injury may account for the age-related decline in follicle numbers, quality, and damage of oocytes [255]. Estrogen has antioxidant properties, and its decrease during menopause along with the reduced levels of antioxidants around the perimenopausal and menopausal period has been associated with generation of OS. Aging is associated with a decrease in the expression of antioxidant genes in the oocytes [256, 257]. There is decreased expression of SOD1, a gene encoding for the cytosolic copper/zinc superoxide dismutase (Cu/ZnSOD), catalase [258, 259], and decreased levels of GSH and GSH-transferase activity [260]. Apart from this, genes from the thioredoxin family are also down-regulated. Changes in the antioxidant enzymatic pattern also have been found in the follicular environment of periovulatory follicles as well as in granulosa cells of women in advanced reproductive age [259, 261].

Tarin et al. (1995, 1996) [255, 262] documented that oxidative damage accumulates in female
gametes due to extended number of years oocytes remain arrested in resting ovarian follicles while remaining metabolically active. This has been supported by Van Blerkom et al. (2000) [263] who concluded from his study that oocytes may be damaged by the OS produced in the follicular microenvironment during their prolonged stay in the ovary and/or as a consequence of a compromised oxygen supply.

As mentioned earlier, the characteristics of oocyte aging include enhancement in ooplasmic microtubule dynamics (OMD), premature release of cortical granules (CG), and hardening of zona pellucida (ZP) with an increase in ZP dissolution time (ZPDT) [252, 264]. Goud et al. (2008) [265] studied the role of O$_2^-$, H$_2$O$_2$, and hypochlorous acid on these parameters of oocyte aging. These agents caused augmentation of oocyte aging, which was more evident in relatively old oocytes, suggesting compromised antioxidant capacity in aging oocytes. The same group, in their previous studies, has shown that exposure to NO delays oocyte aging and improves the integrity of the mitochonadriaal spindle apparatus in young and old oocytes. They suggested that NO may serve as an atypical antioxidant in oocytes rather than a pro-oxidant [266]. This could be attributed to its ability to scavenge cytotoxic ROS and peroxyl lipid radicals. Nitric oxide shares the phosphodiesterase inhibition and antioxidant properties of caffeine, which is a potent antioxidant [267, 268]. Nitric oxide also cause activation of guanylate cyclase, which leads to an increased production of cGMP [269, 270]. Exposure of young and relatively old oocytes to 8-bromo cGMP significantly diminished ZP dissolution time, CG exocytosis, and OMD. A decrease in NO levels also may be responsible for oocyte aging.

Reactive oxygen species causes peroxidative damage to the macromolecules of the cell including lipids, proteins, and nucleic acids. It causes peroxidation of the polyunsaturated fatty acids in the biological membranes and thereby leads to a change in the activity of membrane enzymes and ion channels, and thus, may affect the normal cellular mechanisms required for the fertilization.

Aging is associated with accumulation of somatic mitochondrial DNA (mtDNA) mutations. Oxidative stress is responsible for causing this mitochondrial dysfunction [271, 272]. Being the main site of ROS production, mitochondria are the first to be affected by ROS-mediated injury, and, once damaged, they release a large amount of ROS in the surrounding environment and result in cell cycle arrest and cell death. The presence of higher levels of mtDNA point mutations and rearrangements [273] may seriously compromise mitochondrial ability to generate ATP, and therefore aged oocytes contain less amount of ATP [263]. By exposing young oocytes to hydrogen peroxide, Zhang and associates (2006) [274] concluded that OS decreased mitochondria-derived ATP, a condition which was responsible in turn for a disassembly of mouse MII oocyte spindles.

Thouas et al. [275] compared the developmental ability of young and aged oocytes following mitochondrial injury. The authors found that blastocyst development declined in both age groups with a sudden increase in decline for aged embryos, thereby suggesting that aged oocytes are more sensitive to mitochondrial damage. Reactive oxygen species also causes alteration in mitochondrial membrane potential. Wilding and associates observed a lower electrical potential at the inner mitochondrial membrane in oocytes from reproductively old women. Reactive oxygen species are known to be responsible for disrupting intracellular calcium homeostasis in oocytes, causing them to age [276]. The reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio is responsible for the impairment of calcium transport and the resultant increase in the cytosolic calcium concentration.

Peroxidative DNA damage results in mutations and altered gene expressions [277, 278]. In addition to these effects, OS has been shown to cause meiotic spindle damage and chromosomal alterations leading to low oocyte quality [277].

Liu et al. (2000) [279] studied the number and quality of oocytes in senescence accelerated mouse (SAM). Their studies suggested that old SAM produces significantly fewer oocytes, and these oocytes exhibit meiotic aberrations, as evidenced by chromosome misalignment over both MI and MII spindles, and in some cases gross disruption of spindle morphology. They also suggested that oocyte meiotic division lacks an effective checkpoint for chromosome alignment and spindle integrity during reproductive aging. This has been supported by LeMaire-Adkins et al. (1997) [280] and Roeder et al. (1997) [281]. They also suggested that mammalian female meiosis lacks an efficient metaphase checkpoint control. Similar chromosomal abnormalities also have been found in human studies. The frequency of chromosome abnormalities in human oocytes increases with maternal age [282, 283]. Increased aberrations in spindle formation and chromosome alignment are associated with aging in humans [284, 285]. Oocyte and embryo aneuploidy increases significantly with age in humans [282, 283]. Tarin et al. (1998) [286] also showed that OS induces disturbances in chromosomal distribution in the metaphase II spindle of mouse oocytes in vitro. The disturbance in chromosome alignment at MI could indicate a predisposition to non-disjunction, supporting the hypothesis that explains the maternal age effect for human aneuploidy [287, 288]. Most aneuploidies
associated with maternal ageing are believed to derive from non-disjunctions and meiotic errors initiated at meiosis I (MI) [284, 285, 289], although the underlying mechanism is not well understood [290, 291].

Oocytes matured in vivo or in vitro are used in various ARTs e.g. IVF, intracytoplasmic sperm injection (ICSI). Oocyte aging significantly affects these procedures [292-295]. Hence, control of oocyte aging processes is highly critical in modern ART. Since OS has been associated with oocyte aging and early onset of menopause, the role of antioxidant supplementation in elderly women needs to be studied. Tarin et al. (1998) [286, 296] showed that antioxidant therapy (supplemental intake of vitamin C and vitamin E) was helpful in countering the effects of diamide, a thiol containing oxidizing agent and maternal aging on the meiotic division of chromosomal segregation in mouse oocytes. Later on Tarin et al. (2002) [297] studied the effect of early and late onset administration of oral antioxidants on number and quality of oocytes retrieved from aged mice after exogenous ovarian stimulation. They showed that the administration of oral antioxidant to mice starting either at weaning or at 32 weeks can counteract, at least in part, the negative effects of female aging on the ovarian oocytes. Antioxidant diet can be helpful in preventing oocyte senescence and resulting premature aging. Late administration of oral antioxidants is not as effective as early administration. He also indicated that oral antioxidants may have a role in protecting the hypothalamic-pituitary system from free radical damage. In doing so, baseline levels of LH and FSH are maintained for longer period of time and hence help to delay aging. Murray et al. [298] showed a preventive role of ascorbic acid, SOD, NAC, and catalase in spontaneous induction of granulosa cell apoptosis in a defined serum-free culture system of mouse. Tilly and Tilly showed the same results on rat follicles [299]. All these studies conclude that dietary supplementation with vitamin C and E may counteract oxidative stress in the oocytes and/or granulosa cells from aging females and thereby decrease the proportion of follicles undergoing follicular atresia. To test whether antioxidants play a major role in delaying oocyte aging, Goud et al. investigated the effects of NADPH in modulating OS and oocyte aging in both young and old mouse oocytes. Oocytes that were microinjected with NADPH showed a significant decrease in OMD, indicating a significant potential preventive role of NADPH in oocyte aging (P. Goud, unpublished results). All these are animal studies, and they cannot be applied to humans. The dose of vitamins used in some of these studies falls in the toxic range in humans. An organized and well-monitored study to explore the effectiveness and the safety dosage for antioxidants in humans is needed.

Since NO has been shown to delay oocyte aging, NO supplementation could be used to prevent oocyte aging, e.g., in ART and stem cell research to improve fertility and reproductive outcome in general [266].

It has been proposed that hormone replacement therapy (HRT) has effects on antioxidant levels. Unfer and associates [300] studied the effect of HRT on various antioxidant levels. They observed that HRT antagonizes the decrease in SOD activity that occurs after menopause. Accordingly, lower lipid peroxidation and higher erythrocyte reduced glutathione (GSH) were found in postmenopausal women with HRT when compared to those without HRT [301-303], suggesting that HRT is beneficial as protection against oxidative stress. However, no beneficial effect was found on lipid peroxidation in their study. In addition to these strategies oocyte cryopreservation can be used to preserve fertility.

Conclusions

Oxidative stress does play a role in a number of female reproductive pathologies and strategies to overcome their harmful effects on the female reproductive system can help in preventing or managing many of the reproductive pathologies. The routine use of antioxidants in the management of female reproductive pathologies cannot be justified. Although large trials with oral antioxidants have shown lack of effectiveness in many diseases like preeclampsia, and thus their use in the routine management of many other reproductive pathologies still remains questionable. Even though a promising management option, further studies are warranted to ensure the safety and efficacy of the antioxidants supplementation in many of these disorders.

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