Antioxidants and female reproductive pathologies

Sajal Gupta, Nilopher Surti, Lauren Metterle, Anjali Chandra, Ashok Agarwal

Center for Reproductive Medicine, OB-GYN and Women's Health Institute and Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, Ohio, USA

Submitted: 14 October 2008
Accepted: 28 November 2008

Arch Med Sci 2009; 5, 1A: S151–S173

Corresponding author:
Sajal Gupta, MD
Department of Obstetrics and Gynecology
Cleveland Clinic
9500 Euclid Avenue, Desk A 19.1
Cleveland OH 44115
Phone: 216 444 2240
Fax: 216 445 5526
E-mail: guptas2@ccf.org

Abstract

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, hydaditiform 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.

Key words: recurrent pregnancy loss, preeclampsia, hydatidiform mole, endometriosis, hydrosalpinx, unexplained infertility, premature ovarian aging.

Introduction

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_2O_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, lipoperoxides, 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 erythematous (SLE). Anti-phospholipid 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 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 syncytia 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 placentation [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 antigenic 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 8-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 H_2O_2 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 B_6 (pyridoxal 5-phosphate) is a cofactor in the transsulfuration of homocysteine by causing conversion of cystathionine to cysteine. Hyperhomocysteinaemia results either due to a hereditary defect within the methionine-homocysteine pathway, or it might be acquired as a result of deficiencies of vitamin B_{12}, folate (B_9) [60] and vitamin B_6 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], hyperhomocystenemia 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_6, 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 pre-eclampsia 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 placental 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 peroxynitirite, 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 pre-eclampsia [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_2O_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 and N-acetylcysteine [122, 123]. The preeclamptic plasma activates NF-κB in endothelial cells, which then induces an up-regulation of ICAM-1. Antioxidants were found to inhibit both from happening. Based on the results of this study, antioxidant therapy would seem to be a logical approach to resolving and preventing preeclampsia; however, after they completed a large follow-up study of 2410 women, researchers found that antioxidants did not significantly decrease preeclampsia incidence.

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 premature 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 villous 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-α, IL-1 and IL-6. Increased expression of IL-1β is associated with persistence of the disease and invasion in CHM [145]. Marked elevations of serum IL-1β, IL-6, and TNF-α are associated with progressive (high-risk) GTD, whereas only slight elevation of IL-6 and normal levels of IL-1 and TNF-α 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

syncytiotrophoblasts, considerably stronger in CHM [151-153]. Harma et al. also has shown a dramatic increase in the level of caspase-3 activity in patients with CHM.

As OS has a role in the pathogenesis of the disease, supplementation with antioxidant vitamins such as vitamin C and E could be considered as a treatment option [134].

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 endometrial tissue increases the risk for endometriosis. In a woman's pelvic cavity, the uterus, fallopian tubes, and ovaries are surrounded by peritoneal fluid. It is a major element controlling the peritoneal environment, influencing the development and progression of endometriosis. Thus, studies analyze the peritoneal fluid to find the causative factor for endometriosis because any change in the fluid environment can affect fertilization [160-162].

Retrograde menstruation is common among all women, therefore, it is not clear why some women with retrograde menstruation may develop endometriosis and others do not. Some have suggested that it is the result of a presence of macrophages, iron, environmental contaminants, or refluxed menstrual components like erythrocytes, cell debris, and inflammatory cells that can induce OS [163]. Gutteridge suggests that retrograde menstruation allows for the release of iron from hemoglobin [164]. Iron facilitates the formation of O_2^- and the OH radical, which damage proteins, carbohydrates, nucleotides, and lipids leading to tissue damage and adhesions [163, 165]. The inflammatory process also is believed to be a factor triggering endometriosis, and since free radical metabolism is closely related to this process, this may be a further evidence of OS involvement in the disease [158]. In pelvic endometriosis high numbers of macrophages are present, which are activated in response to endometrial tissue and retrograde 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

criteria, selection of OS markers, and the medium in which OS is measured [166].

Stemming from the possibility that OS is a causative factor in endometriosis, many believe that in addition to higher levels or ROS, women with endometriosis have low levels of antioxidants and even believe that antioxidant supplementation may be beneficial to these women. Fayouzi et al. have concluded from their study that high levels of various antioxidants inhibit the proliferation of endometrial stromal cells. Without sufficient antioxidants, an increase in ROS may induce the excessive proliferation of endometrial tissue, ultimately resulting in endometriosis [100]. Many have found that women with the disease do in fact have low levels of peritoneal fluid antioxidants [175-177, 180, 188]. These women are found to have abnormal expressions of antioxidant enzymes, like glutathione peroxidase and xanthine oxidase [180, 189]. Additionally, Vinatier et al. found significantly reduced levels of the antioxidant vitamin E in the peritoneal fluid of endometriosis patients [190]. Szczepanska et al. , however, noted lower levels of SOD [181]. These data may provide evidence for the role of OS in endometriosis.

However, there are many contradictory findings. Ho et al. reported no difference in TAS production between patients with and without the disease [184]. Others have had similar results [89, 161, 183-185]. Polak et al. conducted a study on 66 women, 18 of whom exhibited minimal or mild endometriosis and also found no difference in the TAS when compared with controls [161]. Jackson et al. specifically reported that antioxidants like vitamin A, lycopene, β-carotene, and vitamin E were not associated with endometriosis [166].

With the potential role of OS in the development of endometriosis, the use of antioxidants in its prevention is being investigated. Studies with animals have found that injection of antioxidant enzymes such as SOD or catalase into the peritoneal cavity prevents the formation of intraperitoneal adhesions at typical endometriotic implantation sites [178]. Many even believe that their use in women with the disease may minimize the extent of lesions and reduce the severity of symptoms and complications [191]. Parazzini et al. reported a significant reduction in the risk for endometriosis in women with a higher intake of green vegetables and fresh fruit, important sources of antioxidants [192]. To explore this further, Mier-Cabrera et al. [191] conducted a randomized double blind trial of vitamin C and E supplementation with women diagnosed with the disease. Overall, they found a decrease in lipid hydroperoxides and MDA in the peripheral blood of these women after 4 months of daily supplementation. These lipid hydroperoxides are products of the peroxidation of unsaturated fatty acids and cholesterol present in LDL, and their presence serves as an indication that OS is affecting the peripheral compartment [193]. In comparison to peritoneal fluid baselines, they speculated that the vitamin intake would also influence the peritoneal environment as well, decreasing oxidative stress. Despite these changes in OS markers, Mier-Cabrera et al. did not find a significant difference in pregnancy rates between the supplementation group and controls [191].

The debate about the role of OS and antioxidants in the development of endometriosis continues, but other possible factors have arisen from these studies as well. Interestingly, many patients with endometriosis exhibit increased peritoneal fluid volume when compared with controls. Since sperm motility is negatively correlated with peritoneal fluid volume, the increased volume possibly may be a causative factor in endometriosis-related infertility [185, 194]. Ho et al. had similar findings [184]. Others have also found that the increased levels of activated macrophages in the peritoneal fluid release TNF-α, inducing toxic effects on gametes [195]. Taketani et al. even reported that the peritoneal fluid of women with endometriosis inhibited the cleavage of two-cell embryos [196]. Whatever the case may be, the exact cause of endometriosis and its associated infertility remains elusive.

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]. 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

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 salphingitis [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 occured 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

Laproscopic 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

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 cystine 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, Kleinveld et al. [238] reported that NAC may act as a pro-oxidant 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 overcomes 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_2O_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 microtubular 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 ion 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 counteracting 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.

References

1. Halliwell B, Gutteridge JM. The definition and measurement of antioxidants in biological systems. Free Radic Biol Med 1995; 18: 125-6.
2. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. N Engl J Med 1988; 319: 189-94.
3. Tallia AF, Cardone DA, Howarth DF, et al. Swanson's Family Practice Review. St. Louis, MO: Mosby; 2005.
4. Fait V, Sela S, Ophir E, et al. Peripheral polymorphonuclear leukocyte priming contributes to oxidative stress in early pregnancy. J Soc Gynecol Investig 2005; 12: 46-9.
5. Safronova VG, Matveeva NK, Avkhacheva NV, Sidel'nikova VM, Van'ko LV, Sukhikh GT. Changes in regulation of oxidase activity of peripheral blood granulocytes in women with habitual abortions. Bull Exp Biol Med 2003; 136: 257-60.
6. Simsek M, Naziroglu M, Simsek H, Cay M, Aksakal M, Kumru S. Blood plasma levels of lipoperoxides, glutathione peroxidase, beta carotene, vitamin A and E in women with habitual abortion. Cell Biochem Funct 1998; 16: 227-31.

7. Miller H, Wilson R, Jenkins C, MacLean MA, Roberts J, Walker JJ. Glutathione levels and miscarriage. Fertil Steril 2000; 74: 1257-8.
8. Vural P, Akgul C, Yildirim A, Canbaz M. Antioxidant defence in recurrent abortion. Clin Chim Acta 2000; 295: 169-77.
9. Nicotra M, Muttinelli C, Sbracia M, Rolfi G, Passi S. Blood levels of lipids, lipoperoxides, vitamin E and glutathione peroxidase in women with habitual abortion. Gynecol Obstet Invest 1994; 38: 223-6.
10. Foidart JM, Hustin J, Dubois M, Schaaps JP. The human placenta becomes haemochorial at the 13th week of pregnancy. Int J Dev Biol 1992; 36: 451-3.
11. Rodesch F, Simon P, Donner C, Jauniaux E. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet Gynecol 1992; 80: 283-5.
12. Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ. Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. Am J Pathol 2000; 157: 2111-22.
13. Jauniaux E, Jurkovic D, Campbell S, Hustin J. Doppler ultrasonographic features of the developing placental circulation: Correlation with anatomic findings. Am J Obstet Gynecol 1992; 166: 585-7.
14. Kliman HJ. Uteroplacental blood flow. The story of decidualization, menstruation, and trophoblast invasion. Am J Pathol 2000; 157: 1759-68.
15. Jaffe R, Woods JR Jr. Color Doppler imaging and in vivo assessment of the anatomy and physiology of the early uteroplacental circulation. Fertil Steril 1993; 60: 293-7.
16. Hung TH, Skepper JN, Burton GJ. In vitro ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. Am J Pathol 2001; 159: 1031-43.
17. Hempstock J, Jauniaux E, Greenwold N, Burton GJ. The contribution of placental oxidative stress to early pregnancy failure. Hum Pathol 2003; 34: 1265-75.
18. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986; 93: 1049-59.
19. Jaffe R, Warsof SL. Color Doppler imaging in the assessment of uteroplacental blood flow in abnormal first trimester intrauterine pregnancies. An attempt to define etiologic mechanisms. J Ultrasound Med 1992; 11: 41-4.
20. Jauniaux E, Greenwold N, Hempstock J, Burton GJ. Comparison of ultrasonographic and Doppler mapping of the intervillous circulation in normal and abnormal early pregnancies. Fertil Steril 2003; 79: 100-6.
21. Jauniaux E, Zaidi J, Jurkovic D, Campbell S, Hustin J. Comparison of colour Doppler features and pathological findings in complicated early pregnancy. Hum Reprod 1994; 9: 2432-7.
22. Kurjak A, Kupesic S. Doppler assessment of the intervillous blood flow in normal and abnormal early pregnancy. Obstet Gynecol 1997; 89: 252-6.
23. Watson AL, Skepper JN, Jauniaux E, Burton GJ. Susceptibility of human placental syncytiotrophoblastic mitochondria to oxygen-mediated damage in relation to gestational age. J Clin Endocrinol Metab 1998; 83: 1697-705.
24. Palmer ME, Watson AL, Burton GJ. Morphological analysis of degeneration and regeneration of syncytiotrophoblast in first trimester placental villi during organ culture. Hum Reprod 1997; 12: 379-82.
25. Jauniaux E, Hempstock J, Greenwold N, Burton GJ. Trophoblastic oxidative stress in relation to temporal and regional differences in maternal placental blood flow in normal and abnormal early pregnancies. Am J Pathol 2003; 162: 115-25.
26. Regan L, Rai R. Thrombophilia and pregnancy loss. J Reprod Immunol 2002; 55: 163-80.
27. Ginsburg KS, Liang MH, Newcomer L, et al. Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis. Ann Intern Med 1992; 117: 997-1002.
28. Cervera R, Khamashta MA, Shoenfeld Y, et al. Morbidity and mortality in the antiphospholipid syndrome during a 5-year period: a multicenter prospective study of 1,000 patients. Ann Rheum Dis 2008 Epub ahead of print.
29. Out HJ, Kooijman CD, Bruinse HW, Derksen RH. Histopathological findings in placentae from patients with intra-uterine fetal death and anti-phospholipid antibodies. Eur J Obstet Gynecol Reprod Biol 1991; 41: 179-86.
30. Sebire NJ, Fox H, Backos M, Rai R, Paterson C, Regan L. Defective endovascular trophoblast invasion in primary antiphospholipid antibody syndrome-associated early pregnancy failure. Hum Reprod 2002; 17: 1067-71.
31. Sebire NJ, Rees H, Paradinas F, et al. Extravillus endovascular implantation site trophoblast invasion is abnormal in complete versus partial molar pregnancies. Placenta 2001; 22: 725-8.
32. Di Simone N, Meroni PL, de Papa N, et al. Anti-phospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered beta2-glycoprotein I. Arthritis Rheum 2000; 43: 140-50.
33. Castellucci M, De Matteis R, Meisser A, et al. Leptin modulates extracellular matrix molecules and metalloproteinases: possible implications for trophoblast invasion. Mol Hum Reprod 2000; 6: 951-8.
34. Di Simone N, Castellani R, Caliandro D, Caruso A. Antiphospholid antibodies regulate the expression of trophoblast cell adhesion molecules. Fertil Steril 2002; 77: 805-11.
35. Francis J, Rai R, Sebire NJ, et al. Impaired expression of endometrial differentiation markers and complement regulatory proteins in patients with recurrent pregnancy loss associated with antiphospholipid syndrome. Mol Hum Reprod 2006; 12: 435-42.
36. Bose P, Black S, Kadyrov M, et al. Adverse effects of lupus anticoagulant positive blood sera on placental viability can be prevented by heparin in vitro. Am J Obstet Gynecol 2004; 191: 2125-31.
37. Matalon ST, Shoenfeld Y, Blank M, Yacobi S, von Landenberg P, Ornoy A. Antiphosphatidylserine antibodies affect rat yolk sacs in culture: a mechanism for fetal loss in antiphospholipid syndrome. Am J Reprod Immunol 2004; 51: 144-51.
38. Girardi G, Berman J, Redecha P, et al. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. J Clin Invest 2003; 112: 1644-54.
39. Girardi G, Salmon JB. The role of complement in pregnancy and fetal loss. Autoimmunity 2003; 36: 19-26.
40. Berman J, Girardi G, Salmon JE. TNF-alpha is a critical effector and a target for therapy in antiphospholipid antibody-induced pregnancy loss. J Immunol 2005; 174: 485-90.
41. Holers VM, Girardi G, Mo L, et al. Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. J Exp Med 2002; 195: 211-20.
42. Horkko S, Miller E, Dudl E, et al. Antiphospholipid antibodies are directed against epitopes of oxidized phospholipids. Recognition of cardiolipin by monoclonal antibodies to epitopes of oxidized low density lipoprotein. J Clin Invest 1996; 98: 815-25.

43. Liu Q, Kobayashi K, Furukawa J, et al. Omega-carboxyl variants of 7-ketocholesteryl esters are ligands for beta(2)-glycoprotein I and mediate antibody-dependent uptake of oxidized LDL by macrophages. J Lipid Res 2002; 43: 1486-95.
44. Lopez LR, Buckner TR, Hurley BL, Kobayashi K, Matsuura E. Determination of oxidized low-density lipoproteins (ox-LDL) versus ox-LDL/beta2GPI complexes for the assessment of autoimmune-mediated atherosclerosis. Ann N Y Acad Sci 2007; 1109: 303-10.
45. Lopez LR, Simpson DF, Hurley BL, Matsuura E. OxLDL/beta2GPI complexes and autoantibodies in patients with systemic lupus erythematosus, systemic sclerosis, and antiphospholipid syndrome: pathogenic implications for vascular involvement. Ann N Y Acad Sci 2005; 1051: 313-22.
46. Redecha P, Tilley R, Tencati M, et al. Tissue factor: a link between C5a and neutrophil activation in anti-phospholipid antibody induced fetal injury. Blood 2007; 110: 2423-31.
47. Krabbendam I, Dekker GA. Pregnancy outcome in patients with a history of recurrent spontaneous miscarriages and documented thrombophilias. Gynecol Obstet Invest 2004; 57: 127-31.
48. Alvarez JG. DNA fragmentation in human spermatozoa: significance in the diagnosis and treatment of infertility. Minerva Ginecol 2003; 55: 233-9.
49. Dalzell LH, McVicar CM, McClure N, Lutton D, Lewis SE. Effects of short and long incubations on DNA fragmentation of testicular sperm. Fertil Steril 2004; 82: 1443-5.
50. Ollero M, Gil-Guzman E, Lopez MC, et al. Characterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis and treatment of male infertility. Hum Reprod 2001; 16: 1912-21.
51. Steele EK, McClure N, Maxwell RJ, Lewis SE. A comparison of DNA damage in testicular and proximal epididymal spermatozoa in obstructive azoospermia. Mol Hum Reprod 1999; 5: 831-5.
52. Carrell DT, Liu L, Peterson CM, et al. Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. Arch Androl 2003; 49: 49-55.
53. Rubes J, Selevan SG, Evenson DP, et al. Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality. Hum Reprod 2005; 20: 2776-83.
54. Liu CH, Tsao HM, Cheng TC, et al. DNA fragmentation, mitochondrial dysfunction and chromosomal aneuploidy in the spermatozoa of oligoasthenoteratozoospermic males. J Assist Reprod Genet 2004; 21: 119-26.
55. Suganuma R, Yanagimachi R, Meistrich ML. Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI. Hum Reprod 2005; 20: 3101-8.
56. Hague WM. Homocysteine and pregnancy. Best Pract Res Clin Obstet Gynaecol 2003; 17: 459-69.
57. Boot MJ, Steegers-Theunissen RP, Poelmann RE, Van Iperen L, Lindemans J, Gittenberger-de Groot AC. Folic acid and homocysteine affect neural crest and neuroepithelial cell outgrowth and differentiation in vitro. Dev Dyn 2003; 227: 301-8.
58. Steegers-Theunissen RP, Boers GH, Blom HJ, Trijbels FJ, Eskes TK. Hyperhomocysteinaemia and recurrent spontaneous abortion or abruptio placentae. Lancet 1992; 339: 1122-3.
59. Nelen WL, Blom HJ, Steegers EA, den Heijer M, Thomas CM, Eskes TK. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. Obstet Gynecol 2000; 95: 519-24.
60. Ray JG, Laskin CA. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: A systematic review. Placenta 1999; 20: 519-29.
61. Wouters MG, Boers GH, Blom HJ, et al. Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. Fertil Steril 1993; 60: 820-5.
62. Del Bianco A, Maruotti G, Fulgieri AM, et al. Recurrent spontaneous miscarriages and hyperhomocysteinemia. Minerva Ginecol 2004; 56: 379-83.
63. Kumar KS, Govindaiah V, Naushad SE, Devi RR, Jyothy A. Plasma homocysteine levels correlated to interactions between folate status and methylene tetrahydrofolate reductase gene mutation in women with unexplained recurrent pregnancy loss. J Obstet Gynaecol 2003; 23: 55-8.
64. Nelen WL, van der Molen EF, Blom HJ, Heil SG, Steegers EA, Eskes TK. Recurrent early pregnancy loss and genetic-related disturbances in folate and homocysteine metabolism. Br J Hosp Med 1997; 58: 511-3.
65. Quere I, Bellet H, Hoffet M, Janbon C, Mares P, Gris JC. A woman with five consecutive fetal deaths: case report and retrospective analysis of hyperhomocysteinemia prevalence in 100 consecutive women with recurrent miscarriages. Fertil Steril 1998; 69: 152-4.
66. de Bree A, Verschuren WM, Blom HJ, Kromhout D. Lifestyle factors and plasma homocysteine concentrations in a general population sample. Am J Epidemiol 2001; 154: 150-4.
67. Sutterlin M, Bussen S, Ruppert D, Steck T. Serum levels of folate and cobalamin in women with recurrent spontaneous abortion. Hum Reprod 1997; 12: 2292-6.
68. Quere I, Mercier E, Bellet H, Janbon C, Mares P, Gris JC. Vitamin supplementation and pregnancy outcome in women with recurrent early pregnancy loss and hyperhomocysteinemia. Fertil Steril 2001; 75: 823-5.
69. Bennett M. Vitamin B12 deficiency, infertility and recurrent fetal loss. J Reprod Med 2001; 46: 209-12.
70. Candito M, Magnaldo S, Bayle J, et al. Clinical B12 deficiency in one case of recurrent spontaneous pregnancy loss. Clin Chem Lab Med 2003; 41: 1026-7.
71. Nadir Y, Hoffman R, Brenner B. Association of homocysteine, vitamin B12, folic acid, and MTHFR C677T in patients with a thrombotic event or recurrent fetal loss. Ann Hematol 2007; 86: 35-40.
72. Carmel R, Green R, Jacobsen DW, Rasmussen K, Florea M, Azen C. Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities. Am J Clin Nutr 1999; 70: 904-10.
73. Carmel R, Rasmussen K, Jacobsen DW, Green R. Comparison of the deoxyuridine suppression test with serum levels of methylmalonic acid and homocysteine in mild cobalamin deficiency. Br J Haematol 1996; 93: 311-8.
74. Stabler SP, Allen RH, Savage DG, Lindenbaum J. Clinical spectrum and diagnosis of cobalamin deficiency. Blood 1990; 76: 871-81.
75. Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ. Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. Am J Clin Nutr 1993; 57: 47-53.
76. Hall CA, Chu RC. Serum homocysteine in routine evaluation of potential vitamin B12 and folate deficiency. Eur J Haematol 1990; 45: 143-9.
77. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in

methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996; 93: 7-9.
78. Schmitz C, Lindpaintner K, Verhoef P, Gaziano JM, Buring J. Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction. A case-control study. Circulation 1996; 94: 1812-4.
79. Oger E, Lacut K, Le Gal G, et al. Hyperhomocysteinemia and low B vitamin levels are independently associated with venous thromboembolism: results from the EDITH study: a hospital-based case-control study. J Thromb Haemost 2006; 4: 793-9.
80. Al-Kunani AS, Knight R, Haswell SJ, Thompson JW, Lindow SW. The selenium status of women with a history of recurrent miscarriage. BJOG 2001; 108: 1094-7.
81. Nicoll AE, Norman J, Macpherson A, Acharya U. Association of reduced selenium status in the aetiology of recurrent miscarriage. Br J Obstet Gynaecol 1999; 106: 1188-91.
82. Kocak I, Aksoy E, Ustun C. Recurrent spontaneous abortion and selenium deficiency. Int J Gynaecol Obstet 1999; 65: 79-80.
83. Kumar KS, Kumar A, Prakash S, Swamy K, Jagadeesan V, Jyothy A. Role of red cell selenium in recurrent pregnancy loss. J Obstet Gynaecol 2002; 22: 181-3.
84. Zaken V, Kohen R, Ornoy A. Vitamins C and E improve rat embryonic antioxidant defense mechanism in diabetic culture medium. Teratology 2001; 64: 33-44.
85. Peng Y, Kwok KH, Yang PH, et al. Ascorbic acid inhibits ROS production, NF-kappa B activation and prevents ethanol-induced growth retardation and microencephaly. Neuropharmacology 2005; 48: 426-34.
86. Olson SE, Seidel GE Jr. Culture of in vitro-produced bovine embryos with vitamin E improves development in vitro and after transfer to recipients. Biol Reprod 2000; 62: 248-52.
87. Crha I, Hruba D, Ventruba P, Fiala J, Totusek J, Visnova H. Ascorbic acid and infertility treatment. Cent Eur J Public Health 2003; 11: 63-7.
88. Rumbold A, Middleton P, Crowther CA. Vitamin supplementation for preventing miscarriage. Cochrane Database Syst Rev 2005: CD004073.
89. Adiga U, D'Souza V, Kamath A, Mangalore N. Antioxidant activity and lipid peroxidation in preeclampsia. J Chin Med Assoc 2007; 70: 435-8.
90. Dordevic NZ, Babic GM, Markovic SD, et al. Oxidative stress and changes in antioxidative defense system in erythrocytes of preeclampsia in women. Reprod Toxicol 2008; 25: 213-8.
91. Mehendale S, Kilari A, Dangat K, Taralekar V, Mahadik S, Joshi S. Fatty acids, antioxidants, and oxidative stress in pre-eclampsia. Int J Gynaecol Obstet 2008; 100: 234-8.
92. Rumiris D, Purwosunu Y, Wibowo N, Farina A, Sekizawa A. Lower rate of preeclampsia after antioxidant supplementation in pregnant women with low antioxidant status. Hypertens Pregn 2006; 25: 241-53.
93. Ryu S, Huppmann AR, Sambangi N, Takacs P, Kauma SW. Increased leukocyte adhesion to vascular endothelium in preeclampsia is inhibited by antioxidants. Am J Obstet Gynecol 2007; 196: 400.e1-7.
94. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000; 183: S1-S22.
95. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet 2002; 77: 67-75.
96. King JF, Slaytor EK, Sullivan EA. Maternal deaths in Australia, 1997-1999. Med J Austr 2004; 181: 413-4.
97. Roberts JM (editor). Pregnancy Related Hypertension. 2nd ed. Philadelphia: WB Saunders, 1989.
98. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 1989; 161: 1200-4.
99. Sibia B (editor). Hypertension in Pregnancy. 3rd ed. New York: Churchilll Livingstone; 1996.
100. Foyouzi N, Berkkanoglu M, Arici A, Kwintkiewicz J, Izquierdo D, Duleba AJ. Effects of oxidants and antioxidants on proliferation of endometrial stromal cells. Fertil Steril 2004; 82 Suppl 3: 1019-22.
101. Kramer MS, Seguin L, Lydon J, Goulet L. Socio-economic disparities in pregnancy outcome: why do the poor fare so poorly? Paediatr Perinatal Epidemiol 2000; 14: 194-210.
102. Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS. Vitamins C and E and the risks of preeclampsia and perinatal complications. N Engl J Med 2006; 354: 1796-806.
103. Roberts JM, Redman CW. Pre-eclampsia: more than pregnancy-induced hypertension. Lancet 1993; 341: 1447-51.
104. Roberts JM, Taylor RN, Goldfien A. Endothelial cell activation as a pathogenetic factor in preeclampsia. Semin Perinatol 1991; 15: 86-93.
105. Perkins AV. Endogenous anti-oxidants in pregnancy and preeclampsia. Aust N Z J Obstet Gynaecol 2006; 46: 77-83.
106. Takehara Y, Yoshioka T, Sasaki J. Changes in the levels of lipoperoxide and antioxidant factors in human placenta during gestation. Acta Med Okayama 1990; 44: 103-11.
107. Madazli R, Benian A, Aydin S, Uzun H, Tolun N. The plasma and placental levels of malondialdehyde, glutathione and superoxide dismutase in pre-eclampsia. J Obstet Gynaecol 2002; 22: 477-80.
108. Mahadik KV, Sina SA. Study of serum levels of superoxide dismutase in preeclampsia and eclampsia: role of the test as a predictive tool. J Obstetr Gynaecol Res 2003; 29: 262-7.
109. Vanderlelie J, Venardos K, Clifton VL, Gude NM, Clarke FM, Perkins AV. Increased biological oxidation and reduced anti-oxidant enzyme activity in pre-eclamptic placentae. Placenta 2005; 26: 53-8.
110. Wang Y, Walsh SW. Antioxidant activities and mRNA expression of superoxide dismutase, catalase, and glutathione peroxidase in normal and preeclamptic placentas. J Soc Gynecol Investig 1996; 3: 179-84.
111. Hubel CA, McLaughlin MK, Evans RW, Hauth BA, Sims CJ, Roberts JM. Fasting serum triglycerides, free fatty acids, and malondialdehyde are increased in preeclampsia, are positively correlated, and decrease within 48 hours post partum. Am J Obstet Gynecol 1996; 174: 975-82.
112. Mikhail MS, Anyaegbunam A, Garfinkel D, Palan PR, Basu J, Romney SL. Preeclampsia and antioxidant nutrients: decreased plasma levels of reduced ascorbic acid, alpha-tocopherol, and beta-carotene in women with preeclampsia. Am J Obstet Gynecol 1994; 171: 150-7.
113. Serdar Z, Gur E, Colakoethullary M, Develioethlu O, Sarandol E. Lipid and protein oxidation and antioxidant function in women with mild and severe preeclampsia. Arch Gynecol Obstetr 2003; 268: 19-25.
114. Maziere C, Dantin F, Conte MA, et al. Polyunsaturated fatty acid enrichment enhances endothelial cell-induced low-density-lipoprotein peroxidation. Biochem J 1998; 336: 57-62.
115. Turpeinen AM, Basu S, Mutanen M. A high linoleic acid diet increases oxidative stress in vivo and affects nitric oxide metabolism in humans. Prostaglandins Leukot Essent Fatty Acids 1998; 59: 229-33.

116. Yanik FF, Amanvermez R, Yanik A, Celik C, Kökçü A. Pre-eclampsia associated with increased lipid peroxidation and decreased serum vitamin E levels. Int J Gynaecol Obstet 1999; 64: 27-33.
117. Lynch RE, Fridovich I. Permeation of the erythrocyte stroma by superoxide radical. J Biolog Chem 1978; 253: 4697-9.
118. Tsukimori K, Fukushima K, Tsushima A, Nakano H. Generation of reactive oxygen species by neutrophils and endothelial cell injury in normal and preeclamptic pregnancies. Hypertensions 2005; 46: 696-700.
119. Winterbourn CC, Stern A. Human red cells scavenge extracellular hydrogen peroxide and inhibit formation of hypochlorous acid and hydroxyl radical. J Clin Invest 1987; 80: 1486-91.
120. Halliwell B, Gutteridge JM (editors). Free radicals in biology and medicine. Oxford: Clarendon Press; 1999.
121. Dargel R. Lipid peroxidation – a common pathogenic mechanism? Exp Toxicol Pathol 1991; 44: 169-81.
122. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med 1999; 222: 222-35.
123. Takacs P, Kauma SW, Sholley MM, Walsh SW, Dinsmoor MJ, Green K. Increased circulating lipid peroxides in severe preeclampsia activate NF-kappaB and upregulate ICAM-1 in vascular endothelial cells. Faseb J 2001; 15: 279-81.
124. Chappell LC, Seed PT, Briley AL, et al. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. Lancet 1999; 354: 810-6.
125. Beazley D, Ahokas R, Livingston J, Griggs M, Sibai BM. Vitamin C and E supplementation in women at high risk for preeclampsia: a double-blind, placebo-controlled trial. Am J Obstet Gynecol 2005; 192: 520-1.
126. Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. Lancet 2006; 367: 1145-54.
127. Saugstad OD. Hypoxanthine as an indicator of hypoxia: its role in health and disease through free radical production. Pediatr Res 1988; 23: 143-50.
128. Saugstad OD. Update on oxygen radical disease in neonatology. Cur Opin Obstetr Gynecol 2001; 13: 147-53.
129. Spinnato JA 2nd, Freire S, Pinto ES, et al. Antioxidant therapy to prevent preeclampsia: a randomized controlled trial. Obstet Gynecol 2007; 110: 1311-8.
130. Jeyabalan A, Caritis SN. Antioxidants and the prevention of preeclampsia – unresolved issues. N Engl J Med 2006; 354: 1841-3.
131. Rumbold A, Crowther CA. Vitamin C supplementation in pregnancy. Cochrane Database Syst Rev 2005; (2): CD004072.
132. Jeffers MD, O'Dwyer P, Curran B, Leader M, Gillan JE. Partial hydatidiform mole: a common but underdiagnosed condition. A 3-year retrospective clinicopathological and DNA flow cytometric analysis. Int J Gynecol Pathol 1993; 12: 315-23.
133. Berkowitz RS, Goldstein DP. Chorionic tumors. N Engl J Med 1996; 335: 1740-8.
134. Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly 2003; 133: 563-6.
135. Harma M, Kocyigit A. Comparison of protein carbonyl and total plasma thiol concentrations in patients with complete hydatidiform mole with those in healthy pregnant women. Acta Obstet Gynecol Scand 2004; 83: 857-60.
136. Walsh SW. The role of fatty acid peroxidation and antioxidant status in normal pregnancy and in pregnancy complicated by preeclampsia. World Rev Nutr Diet 1994; 76: 114-8.
137. Telci A, Cakatay U, Salman S, Satman I, Sivas A. Oxidative protein damage in early stage Type 1 diabetic patients. Diabetes Res Clin Pract 2000; 50: 213-23.
138. Harma M, Kocyigit A, Demir N. Role of plasma nitric oxide in complete hydatidiform mole. Eur J Gynaecol Oncol 2004; 25: 333-5.
139. Harma M. Defective placentation and resultant oxidative stress play a similar role in complete hydatidiform mole to that in preeclampsia and early pregnancy loss. Med Hypotheses 2006; 66: 100-2.
140. Jauniaux E, Hustin J. Histological examination of first trimester spontaneous abortions: the impact of materno-embryonic interface features. Histopathology 1992; 21: 409-14.
141. Montes M, Roberts D, Berkowitz RS, Genest DR. Prevalence and significance of implantation site trophoblastic atypia in hydatidiform moles and spontaneous abortions. Am J Clin Pathol 1996; 105: 411-6.
142. Batorfi J, Fulop V, Kim JH, et al. Osteopontin is down-regulated in hydatidiform mole. Gynecol Oncol 2003; 89: 134-9.
143. Jauniaux E, Burton GJ. Pathophysiology of histological changes in early pregnancy loss. Placenta 2005; 26: 114-23.
144. Kabawat SE, Mostoufi-Zadeh M, Berkowitz RS, Driscoll SG, Goldstein DP, Bhan AK. Implantation site in complete molar pregnancy: a study of immunologically competent cells with monoclonal antibodies. Am J Obstet Gynecol 1985; 152: 97-9.
145. Prabha B, Molykutty J, Swapna A, Rajalekshmi TN, Gangadharan VP. Increased expression of interleukin-1 beta is associated with persistence of the disease and invasion in complete hydatidiform moles (CHM). Eur J Gynaecol Oncol 2001; 22: 50-6.
146. Shaarawy M, Darwish NA. Serum cytokines in gestational trophoblastic diseases. Acta Oncol 1995; 34: 177-82.
147. Shaarawy M, Darwish NA, Abdel-Aziz O. Serum interleukin-2 and soluble interleukin-2 receptor in gestational trophoblastic diseases. J Soc Gynecol Investig 1996; 3: 39-46.
148. Berkowitz RS, Cramer DW, Bernstein MR, Cassells S, Driscoll SG, Goldstein DP. Risk factors for complete molar pregnancy from a case-control study. Am J Obstet Gynecol 1985; 152: 1016-20.
149. Berkowitz RS, Bernstein MR, Harlow BL, et al. Case-control study of risk factors for partial molar pregnancy. Am J Obstet Gynecol 1995; 173: 788-94.
150. Harma M, Kocyigit A, Erel O. Increased DNA damage in patients with complete hydatidiform mole. Mutat Res 2005; 583: 49-54.
151. Fulop V, Mok SC, Genest DR, Szigetvari I, Cseh I, Berkowitz RS. c-myc, c-erbB-2, c-fms and bcl-2 oncoproteins. Expression in normal placenta, partial and complete mole, and choriocarcinoma. J Reprod Med 1998; 43: 101-10.
152. Qiao S, Nagasaka T, Harada T, Nakashima N. p53, Bax and Bcl-2 expression, and apoptosis in gestational trophoblast of complete hydatidiform mole. Placenta 1998; 19: 361-9.
153. Sakuragi N, Matsuo H, Coukos G, et al. Differentiation-dependent expression of the BCL-2 proto-oncogene in the human trophoblast lineage. J Soc Gynecol Investig 1994; 1: 164-72.
154. Wheeler JM. Epidemiology of endometriosis-associated infertility. J Reprod Med 1989; 34: 41-6.

155. Mahmood TA, Templeton A. Prevalence and genesis of endometriosis. Hum Reprod 1991; 6: 544-9.
156. Moen MH, Muus KM. Endometriosis in pregnant and non-pregnant women at tubal sterilization. Hum Reprod 1991; 6: 699-702.
157. Rawson JM. Prevalence of endometriosis in asymptomatic women. J Reprod Med 1991; 36: 513-5.
158. Alpay Z, Saed GM, Diamond MP. Female infertility and free radicals: potential role in adhesions and endometriosis. J Soc Gynecol Investig 2006; 13: 390-8.
159. Berube S, Marcoux S, Langevin M, Maheux R. Fecundity of infertile women with minimal or mild endometriosis and women with unexplained infertility. The Canadian Collaborative Group on Endometriosis. Fertil Steril 1998; 69: 1034-41.
160. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 2003; 79: 829-43.
161. Polak G, Koziol-Montewka M, Gogacz M, Blaszkowska I, Kotarski J. Total antioxidant status of peritoneal fluid in infertile women. Eur J Obstet Gynecol Reprod Biol 2001; 94: 261-3.
162. Polak G, Koziol-Montewka M, Tarkowski R, Kotarski J. Peritoneal fluid and plasma 4-hydroxynonenal and malonyldialdehyde concentrations in infertile women. Ginekol Pol 2001; 72: 1316-20.
163. Arumugam K, Yip YC. De novo formation of adhesions in endometriosis: the role of iron and free radical reactions. Fertil Steril 1995; 64: 62-4.
164. Gutteridge JM. Iron promoters of the Fenton reaction and lipid peroxidation can be released from haemoglobin by peroxides. FEBS Lett 1986; 201: 291-5.
165. MacNee W, Rahman I. Oxidants/antioxidants in idiopathic pulmonary fibrosis. Thorax 1995; 50 Suppl 1: S53-8.
166. Jackson LW, Schisterman EF, Dey-Rao R, Browne R, Armstrong D. Oxidative stress and endometriosis. Hum Reprod 2005; 20: 2014-20.
167. Kyama CM, Debrock S, Mwenda JM, D'Hooghe TM. Potential involvement of the immune system in the development of endometriosis. Reprod Biol Endocrinol 2003; 1: 123.
168. Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. Fertil Steril 2002; 77: 861-70.
169. Osborn BH, Haney AF, Misukonis MA, Weinberg JB. Inducible nitric oxide synthase expression by peritoneal macrophages in endometriosis-associated infertility. Fertil Steril 2002; 77: 46-51.
170. Shanti A, Santanam N, Morales AJ, Parthasarathy S, Murphy AA. Autoantibodies to markers of oxidative stress are elevated in women with endometriosis. Fertil Steril 1999; 71: 1115-8.
171. Agarwal A, Allamaneni SS. Role of free radicals in female reproductive diseases and assisted reproduction. Reprod Biomed Online 2004; 9: 338-47.
172. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol 2005; 3: 28.
173. Allaire C. Endometriosis and infertility: a review. J Reprod Med 2006; 51: 164-8.
174. Curtis P, Lindsay P, Jackson AE, Shaw RW. Adverse effects on sperm movement characteristics in women with minimal and mild endometriosis. Br J Obstet Gynaecol 1993; 100: 165-9.
175. Murphy AA, Palinski W, Rankin S, Morales AJ, Parthasarathy S. Evidence for oxidatively modified lipid-protein complexes in endometrium and endometriosis. Fertil Steril 1998; 69: 1092-4.
176. Murphy AA, Palinski W, Rankin S, Morales AJ, Parthasarathy S. Macrophage scavenger receptor(s) and oxidatively modified proteins in endometriosis. Fertil Steril 1998; 69: 1085-91.
177. Murphy AA, Santanam N, Morales AJ, Parthasarathy S. Lysophosphatidyl choline, a chemotactic factor for monocytes/T-lymphocytes is elevated in endometriosis. J Clin Endocrinol Metab 1998; 83: 2110-3.
178. Portz DM, Elkins TE, White R, Warren J, Adadevoh S, Randolph J. Oxygen free radicals and pelvic adhesion formation: I. Blocking oxygen free radical toxicity to prevent adhesion formation in an endometriosis model. Int J Fertil 1991; 36: 39-42.
179. Halme J, Becker S, Hammond MG, Raj MH, Raj S. Increased activation of pelvic macrophages in infertile women with mild endometriosis. Am J Obstet Gynecol 1983; 145: 333-7.
180. Ota H, Igarashi S, Tanaka T. Xanthine oxidase in eutopic and ectopic endometrium in endometriosis and adenomyosis. Fertil Steril 2001; 75: 785-90.
181. Szczepanska M, Kozlik J, Skrzypczak J, Mikolajczyk M. Oxidative stress may be a piece in the endometriosis puzzle. Fertil Steril 2003; 79: 1288-93.
182. Zeller JM, Henig I, Radwanska E, Dmowski WP. Enhancement of human monocyte and peritoneal macrophage chemiluminescence activities in women with endometriosis. Am J Reprod Immunol Microbiol 1987; 13: 78-82.
183. Arumugam K, Dip YC. Endometriosis and infertility: the role of exogenous lipid peroxides in the peritoneal fluid. Fertil Steril 1995; 63: 198-9.
184. Ho HN, Wu MY, Chen SU, Chao KH, Chen CD, Yang YS. Total antioxidant status and nitric oxide do not increase in peritoneal fluids from women with endometriosis. Hum Reprod 1997; 12: 2810-5.
185. Wang Y, Sharma RK, Falcone T, Goldberg J, Agarwal A. Importance of reactive oxygen species in the peritoneal fluid of women with endometriosis or idiopathic infertility. Fertil Steril 1997; 68: 826-30.
186. Bedaiwy MA, Goldberg JM, Falcone T, et al. Relationship between oxidative stress and embryotoxicity of hydrosalpingeal fluid. Hum Reprod 2002; 17: 601-4.
187. Bedaiwy MA, Falcone T, Sharma RK, et al. Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. Hum Reprod 2002; 17: 426-31.
188. Sugino N, Karube-Harada A, Taketani T, Sakata A, Nakamura Y. Withdrawal of ovarian steroids stimulates prostaglandin F2alpha production through nuclear factor-kappaB activation via oxygen radicals in human endometrial stromal cells: potential relevance to menstruation. J Reprod Develop 2004; 50: 215-25.
189. Ota H, Igarashi S, Kato N, Tanaka T. Aberrant expression of glutathione peroxidase in eutopic and ectopic endometrium in endometriosis and adenomyosis. Fertil Steril 2000; 74: 313-8.
190. Vinatier D, Cosson M, Dufour P. Is endometriosis an endometrial disease? Eur J Obstet Gynecol Reprod Biol 2000; 91: 113-25.
191. Mier-Cabrera J, Genera-Garcia M, De la Jara-Diaz J, Perichart-Perera O, Vadillo-Ortega F, Hernandez-Guerrero C. Effect of vitamins C and E supplementation on peripheral oxidative stress markers and pregnancy rate in women with endometriosis. Int J Gynaecol Obstet 2008; 100: 252-6.
192. Parazzini F, Chiaffarino F, Surace M, et al. Selected food intake and risk of endometriosis. Hum Reprod 2004; 19: 1755-9.

193. Borel P, Mekki N, Boirie Y, et al. Postprandial chylomicron and plasma vitamin E responses in healthy older subjects compared with younger ones. Eur J Clin Investig 1997; 27: 812-21.
194. van Furth R, Raeburn JA, van Zwet TL. Characteristics of human mononuclear phagocytes. Blood 1979; 54: 485-500.
195. Lee KS, Joo BS, Na YJ, Yoon MS, Choi OH, Kim WW. Relationships between concentrations of tumor necrosis factor-alpha and nitric oxide in follicular fluid and oocyte quality. J Assist Reprod Genet 2000; 17: 222-8.
196. Taketani Y, Kuo TM, Mizuno M. Comparison of cytokine levels and embryo toxicity in peritoneal fluid in infertile women with untreated or treated endometriosis. Am J Obstet Gynecol 1992; 167: 265-70.
197. Strandell A. How to treat hydrosalpinges: IVF as the treatment of choice. Reprod Biomed Online 2002; 4 Suppl 3: 37-9.
198. Strandell A, Lindhard A, Waldenstrom U, Thorburn J. Hydrosalpinx and IVF outcome: cumulative results after salpingectomy in a randomized controlled trial. Hum Reprod 2001; 16: 2403-10.
199. Strandell A, Lindhard A, Waldenstrom U, Thorburn J, Janson PO, Hamberger L. Hydrosalpinx and IVF outcome: a prospective, randomized multicentre trial in Scandinavia on salpingectomy prior to IVF. Hum Reprod 1999; 14: 2762-9.
200. Strandell A, Lindhard A. Hydrosalpinx and ART. Salpingectomy prior to IVF can be recommended to a well-defined subgroup of patients. Hum Reprod 2000; 15: 2072-4.
201. Strandell A, Sjogren A, Bentin-Ley U, Thorburn J, Hamberger L, Brannstrom M. Hydrosalpinx fluid does not adversely affect the normal development of human embryos and implantation in vitro. Hum Reprod 1998; 13: 2921-5.
202. Granot I, Dekel N, Segal I, Fieldust S, Shoham Z, Barash A. Is hydrosalpinx fluid cytotoxic? Hum Reprod 1998; 13: 1620-4.
203. Strandell A, Lindhard A. Why does hydrosalpinx reduce fertility? The importance of hydrosalpinx fluid. Hum Reprod 2002; 17: 1141-5.
204. Chan LY, Chiu PY, Cheung LP, Haines CJ, Tung HF, Lau TK. A study of teratogenicity of hydrosalpinx fluid using a whole rat embryo culture model. Hum Reprod 2003; 18: 955-8.
205. Strandell A, Waldenstrom U, Nilsson L, Hamberger L. Hydrosalpinx reduces in-vitro fertilization/embryo transfer pregnancy rates. Hum Reprod 1994; 9: 861-3.
206. Meyer WR, Castelbaum AJ, Somkuti S, et al. Hydrosalpinges adversely affect markers of endometrial receptivity. Hum Reprod 1997; 12: 1393-8.
207. Kubo A, Sasada M, Nishimura T, et al. Oxygen radical generation by polymorphonuclear leucocytes of beige mice. Clin Exp Immunol 1987; 70: 658-63.
208. Chanr LY, Chiu PY, Lau TK. Hydrosalpinx fluid induced embryotoxicity and lipid peroxidation. Reprod Toxicol 2004; 19: 147-8.
209. Morrow JD, Roberts LJ 2nd. Quantification of nonciclooxygenase derived prostanoids as a marker of oxidative stress. Free Radic Biol Med 1991; 10: 195-200.
210. Conaghan J, Handyside AH, Winston RM, Leese HJ. Effects of pyruvate and glucose on the development of human preimplantation embryos in vitro. J Reprod Fertil 1993; 99: 87-95.
211. Dickens CJ, Maguiness SD, Comer MT, Palmer A, Rutherford AJ, Leese HJ. Human tubal fluid: formation and composition during vascular perfusion of the fallopian tube. Hum Reprod 1995; 10: 505-8.
212. Tay JI, Rutherford AJ, Killick SR, Maguiness SD, Partridge RJ, Leese HJ. Human tubal fluid: production, nutrient composition and response to adrenergic agents. Hum Reprod 1997; 12: 2451-6.
213. Illera MJ, Cullinan E, Gui Y, Yuan L, Beyler SA, Lessey BA. Blockade of the alpha(v)beta(3) integrin adversely affects implantation in the mouse. Biol Reprod 2000; 62: 1285-90.
214. Andersen AN, Lindhard A, Loft A, Ziebe S, Andersen CY. The infertile patient with hydrosalpinges – IVF with or without salpingectomy? Hum Reprod 1996; 11: 2081-4.
215. Bloechle M, Schreiner T, Lisse K. Recurrence of hydrosalpinges after transvaginal aspiration of tubal fluid in an IVF cycle with development of a serometra. Hum Reprod 1997; 12: 703-5.
216. Mansour RT, Aboulghar MA, Serour GI, Riad R. Fluid accumulation of the uterine cavity before embryo transfer: a possible hindrance for implantation. J Vitro Fert Embryo Transf 1991; 8: 157-9.
217. Levi AJ, Segars JH, Miller BT, Leondires MP. Endometrial cavity fluid is associated with poor ovarian response and increased cancellation rates in ART cycles. Hum Reprod 2001; 16: 2610-5.
218. Sharara FI, McClamrock HD. Endometrial fluid collection in women with hydrosalpinx after human chorionic gonadotrophin administration: a report of two cases and implications for management. Hum Reprod 1997; 12: 2816-9.
219. MM IJ, Evers JL, Dunselman GA, Volovics L, Hoogland HJ. Relation between endometrial wavelike activity and fecundability in spontaneous cycles. Fertil Steril 1997; 67: 492-6.
220. MM IJ, Hoogland HJ, Dunselman GA, Lo CR, Evers JL. Endometrial wave direction switch and the outcome of in vitro fertilization. Fertil Steril 1999; 71: 476-81.
221. Eytan O, Azem F, Gull I, Wolman I, Elad D, Jaffa AJ. The mechanism of hydrosalpinx in embryo implantation. Hum Reprod 2001; 16: 2662-7.
222. Bredkjaer HE, Ziebe S, Hamid B, et al. Delivery rates after in-vitro fertilization following bilateral salpingectomy due to hydrosalpinges: a case control study. Hum Reprod 1999; 14: 101-5.
223. Nackley AC, Muasher SJ. The significance of hydrosalpinx in vitro fertilization. Fertil Steril 1998; 69: 373-84.
224. Vandromme J, Chasse E, Lejeune B, Van Rysselberge M, Delvigne A, Leroy F. Hydrosalpinges in in-vitro fertilization: an unfavourable prognostic feature. Hum Reprod 1995; 10: 576-9.
225. Mosgaard B, Hertz J, Steenstrup BR, Sorensen SS, Lindhard A, Andersen AN. Surgical management of tubal infertility. A regional study. Acta Obstet Gynecol Scand 1996; 75: 469-4.
226. Goto Y, Noda Y, Mori T, Nakano M. Increased generation of reactive oxygen species in embryos cultured in vitro. Free Radic Biol Med 1993; 15: 69-75.
227. Goto Y, Noda Y, Narimoto K, Umaoka Y, Mori T. Oxidative stress on mouse embryo development in vitro. Free Radic Biol Med 1992; 13: 47-53.
228. Legge M, Sellens MH. Free radical scavengers ameliorate the 2-cell block in mouse embryo culture. Hum Reprod 1991; 6: 867-71.
229. Goldman MB, Barbieri RL. Infertility. In: Women and Health. Goldman MB (editor). San Diego: Academic Press 2000; 196-214.
230. N E. Infertility. In: Cherney A NL, editor. Current Obstetrics and Gynecologic Diagnosis and Treatment. 9 ed. New York: McGaw-Hill 979-1000.

231. Polak G, Koziol-Montewka M, Gogacz M, Kotarski J. Total antioxidant status and activity of an extracellular superoxide dismutase in peritoneal fluid and plasma from women with unexplained infertility [Polish]. Ginekol Pol 2000; 71: 571-6.
232. Storey BT. Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. Mol Hum Reprod 1997; 3: 203-13.
233. Polak G, Rola R, Gogacz M, Koziol-Montewka M, Kotarski J. Malonyldialdehyde and total antioxidant status in the peritoneal fluid of infertile women [Polish]. Ginekol Pol 1999; 70: 135-40.
234. Dong M, Shi Y, Cheng Q, Hao M. Increased nitric oxide in peritoneal fluid from women with idiopathic infertility and endometriosis. J Reprod Med 2001; 46: 887-91.
235. Howard JM, Davies S, Hunnisett A. Red cell magnesium and glutathione peroxidase in infertile women-effects of oral supplementation with magnesium and selenium. Magnes Res 1994; 7: 49-57.
236. Ahola T, Fellman V, Laaksonen R, et al. Pharmacokinetics of intravenous N-acetylcysteine in pre-term new-born infants. Eur J Clin Pharmacol 1999; 55: 645-50.
237. De Mattia G, Bravi MC, Laurenti O, et al. Reduction of oxidative stress by oral N-acetyl-L-cysteine treatment decreases plasma soluble vascular cell adhesion molecule-1 concentrations in non-obese, non-dyslipidaemic, normotensive, patients with non-insulin-dependent diabetes. Diabetologia 1998; 41: 1392-6.
238. Kleinveld HA, Demacker PN, Stalenhoef AF. Failure of N-acetylcysteine to reduce low-density lipoprotein oxidizability in healthy subjects. Eur J Clin Pharmacol 1992; 43: 639-42.
239. Sekhon B, Sekhon C, Khan M, Patel SJ, Singh I, Singh AK. N-Acetyl cysteine protects against injury in a rat model of focal cerebral ischemia. Brain Res 2003; 971: 1-8.
240. Wong M, Wells PG. Effects of N-acetylcysteine on fetal development and on phenytoin teratogenicity in mice. Teratog Carcinog Mutagen 1988; 8: 65-79.
241. Lappas M, Permezel M, Rice GE. N-Acetyl-cysteine inhibits phospholipid metabolism, proinflammatory cytokine release, protease activity, and nuclear factor-kappaB deoxyribonucleic acid-binding activity in human fetal membranes in vitro. J Clin Endocrinol Metab 2003; 88: 1723-9.
242. Badawy A, Baker El Nashar A, El Totongy M. Clomiphene citrate plus N-acetyl cysteine versus clomiphene citrate for augmenting ovulation in the management of unexplained infertility: a randomized double-blind controlled trial. Fertil Steril 2006; 86: 647-50.
243. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002; 8: 141-54.
244. Baird DT, Collins J, Egozcue J, et al. Fertility and ageing. Hum Reprod Update 2005; 11: 261-76.
245. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 1992; 7: 1342-6.
246. Sauer MV, Paulson RJ, Lobo RA. A preliminary report on oocyte donation extending reproductive potential to women over 40. N Engl J Med 1990; 323: 1157-60.
247. Navot D, Bergh PA, Williams MA, et al. Poor oocyte quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet 1991; 337: 1375-7.
248. Igarashi H, Takahashi E, Hiroi M, Doi K. Aging-related changes in calcium oscillations in fertilized mouse oocytes. Mol Reprod Dev 1997; 48: 383-90.
249. Kikuchi K, Naito K, Noguchi J, et al. Maturation/M-phase promoting factor: a regulator of aging in porcine oocytes. Biol Reprod 2000; 63: 715-22.
250. Xu Z, Abbott A, Kopf GS, Schultz RM, Ducibella T. Spontaneous activation of ovulated mouse eggs: time-dependent effects on M-phase exit, cortical granule exocytosis, maternal messenger ribonucleic acid recruitment, and inositol 1,4,5-trisphosphate sensitivity. Biol Reprod 1997; 57: 743-50.
251. Verlhac MH, Kubiak JZ, Clarke HJ, Maro B. Microtubule and chromatin behavior follow MAP kinase activity but not MPF activity during meiosis in mouse oocytes. Development 1994; 120: 1017-25.
252. Goud AP, Goud PT, Van Oostveldt P, Diamond MP, Dhont M. Dynamic changes in microtubular cytoskeleton of human postmature oocytes revert after ooplasm transfer. Fertil Steril 2004; 81: 323-31.
253. Longo FJ. Changes in the zones pellucidae and plasmalemma of aging mouse eggs. Biol Reprod 1981; 25: 399-411.
254. Zernicka-Goetz M, Kubiak JZ, Antony C, Maro B. Cytoskeletal organization of rat oocytes during metaphase II arrest and following abortive activation: a study by confocal laser scanning microscopy. Mol Reprod Dev 1993; 35: 165-75.
255. Tarin JJ. Potential effects of age-associated oxidative stress on mammalian oocytes/embryos. Mol Hum Reprod 1996; 2: 717-24.
256. Bachvarova R. Gene expression during oogenesis and oocyte development in mammals. Dev Biol 1985; 1: 453-524.
257. Hamatani T, Falco G, Carter MG, et al. Age-associated alteration of gene expression patterns in mouse oocytes. Hum Mol Genet 2004; 13: 2263-78.
258. Inal ME, Kanbak G, Sunal E. Antioxidant enzyme activities and malondialdehyde levels related to aging. Clin Chim Acta 2001; 305: 75-80.
259. Tatone C, Carbone MC, Falone S, et al. Age-dependent changes in the expression of superoxide dismutases and catalase are associated with ultrastructural modifications in human granulosa cells. Mol Hum Reprod 2006; 12: 655-60.
260. Tarin JJ, Gomez-Piquer V, Pertusa JF, Hermenegildo C, Cano A. Association of female aging with decreased parthenogenetic activation, raised MPF, and MAPKs activities and reduced levels of glutathione S-transferases activity and thiols in mouse oocytes. Mol Reprod Dev 2004; 69: 402-10.
261. Carbone MC, Tatone C, Delle Monache S, et al. Antioxidant enzymatic defences in human follicular fluid: characterization and age-dependent changes. Mol Hum Reprod 2003; 9: 639-43.
262. Tarin JJ. Aetiology of age-associated aneuploidy: a mechanism based on the 'free radical theory of ageing' Hum Reprod 1995; 10: 1563-5.
263. Van Blerkom J. Intrafollicular influences on human oocyte developmental competence: perifollicular vascularity, oocyte metabolism and mitochondrial function. Hum Reprod 2000; 15 Suppl 2: 173-88.
264. Goud AP, Goud PT, Diamond MP, Van Oostveldt P, Hughes MR. Microtubule turnover in ooplasm biopsy reflects ageing phenomena in the parent oocyte. Reprod Biomed Online 2005; 11: 43-52.
265. Goud AP, Goud PT, Diamond MP, Gonik B, Abu-Soud HM. Reactive oxygen species and oocyte aging: role of superoxide, hydrogen peroxide, and hypochlorous acid. Free Radic Biol Med 2008; 44: 1295-304.

266. Goud AP, Goud PT, Diamond MP, Abu-Soud HM. Nitric oxide delays oocyte aging. Biochemistry 2005; 44: 11361-8.
267. Azam S, Hadi N, Khan NU, Hadi SM. Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. Med Sci Monit 2003; 9: BR325-30.
268. Mukhopadhyay S, Mondal A, Poddar MK. Chronic administration of caffeine: effect on the activities of hepatic antioxidant enzymes of Ehrlich ascites tumor-bearing mice. Indian J Exp Biol 2003; 41: 283-9.
269. Schmidt HH, Walter U. NO at work. Cell 1994; 78: 919-25.
270. Vincent SR. Nitric oxide: a radical neurotransmitter in the central nervous system. Prog Neurobiol 1994; 42: 129-60.
271. Terzioglu M, Larsson NG. Mitochondrial dysfunction in mammalian ageing. Novartis Found Symp 2007; 287: 197-208; discussion -13.
272. Trifunovic A, Larsson NG. Mitochondrial dysfunction as a cause of ageing. J Intern Med 2008; 263: 167-78.
273. Keefe DL, Niven-Fairchild T, Powell S, Buradagunta S. Mitochondrial deoxyribonucleic acid deletions in oocytes and reproductive aging in women. Fertil Steril 1995; 64: 577-83.
274. Zhang X, Wu XQ, Lu S, Guo YL, Ma X. Deficit of mitochondria-derived ATP during oxidative stress impairs mouse MII oocyte spindles. Cell Res 2006; 16: 841-50.
275. Thouas GA, Trounson AO, Jones GM. Effect of female age on mouse oocyte developmental competence following mitochondrial injury. Biol Reprod 2005; 73: 366-73.
276. Takahashi T, Takahashi E, Igarashi H, Tezuka N, Kurachi H. Impact of oxidative stress in aged mouse oocytes on calcium oscillations at fertilization. Mol Reprod Dev 2003; 66: 143-52.
277. Choi WJ, Banerjee J, Falcone T, Bena J, Agarwal A, Sharma RK. Oxidative stress and tumor necrosis factor-alpha-induced alterations in metaphase II mouse oocyte spindle structure. Fertil Steril 2007; 88 (4 Suppl): 1220-31.
278. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 2003; 17: 1195-214.
279. Liu L, Keefe DL. Ageing-associated aberration in meiosis of oocytes from senescence-accelerated mice. Hum Reprod 2002; 17: 2678-85.
280. LeMaire-Adkins R, Radke K, Hunt PA. Lack of checkpoint control at the metaphase/anaphase transition: a mechanism of meiotic nondisjunction in mammalian females. J Cell Biol 1997; 139: 1611-9.
281. Roeder GS. Meiotic chromosomes: it takes two to tango. Genes Dev 1997; 11: 2600-21.
282. Hassold T, Chiu D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. Hum Genet 1985; 70: 11-7.
283. Plachot M, Veiga A, Montagut J, et al. Are clinical and biological IVF parameters correlated with chromosomal disorders in early life: a multicentric study. Hum Reprod 1988; 3: 627-35.
284. Battaglia DE, Goodwin P, Klein NA, Soules MR. Influence of maternal age on meiotic spindle assembly in oocytes from naturally cycling women. Hum Reprod 1996; 11: 2217-22.
285. Volarcik K, Sheean L, Goldfarb J, Woods L, Abdul-Karim FW, Hunt P. The meiotic competence of in-vitro matured human oocytes is influenced by donor age: evidence that folliculogenesis is compromised in the reproductively aged ovary. Hum Reprod 1998; 13: 154-60.
286. Tarin JJ, Vendrell FJ, Ten J, Cano A. Antioxidant therapy counteracts the disturbing effects of diamide and maternal ageing on meiotic division and chromosomal segregation in mouse oocytes. Mol Hum Reprod 1998; 4: 281-8.
287. Crowley PH, Gulati DK, Hayden TL, Lopez P, Dyer R. A chiasma-hormonal hypothesis relating Down's syndrome and maternal age. Nature 1979; 280: 417-8.
288. Henderson SA, Edwards RG. Chiasma frequency and maternal age in mammals. Nature 1968; 218: 22-8.
289. Angell R. First-meiotic-division nondisjunction in human oocytes. Am J Hum Genet 1997; 61: 23-32.
290. Nicolaidis P, Petersen MB. Origin and mechanisms of non-disjunction in human autosomal trisomies. Hum Reprod 1998; 13: 313-9.
291. Petersen MB, Mikkelsen M. Nondisjunction in trisomy 21: origin and mechanisms. Cytogenet Cell Genet 2000; 91: 199-203.
292. Fissore RA, Kurokawa M, Knott J, Zhang M, Smyth J. Mechanisms underlying oocyte activation and postovulatory ageing. Reproduction 2002; 124: 745-54.
293. Goud P, Goud A, Van Oostveldt P, Van der Elst J, Dhont M. Fertilization abnormalities and pronucleus size asynchrony after intracytoplasmic sperm injection are related to oocyte postmaturity. Fertil Steril 1999; 72: 245-52.
294. Goud PT, Goud AP, Laverge H, De Sutter P, Dhont M. Effect of post-ovulatory age and calcium in the injection medium on the male pronucleus formation and metaphase entry following injection of human spermatozoa into golden hamster oocytes. Mol Hum Reprod 1999; 5: 227-33.
295. Tarin JJ, Perez-Albala S, Aguilar A, Minarro J, Hermenegildo C, Cano A. Long-term effects of postovulatory aging of mouse oocytes on offspring: a two-generational study. Biol Reprod 1999; 61: 1347-55.
296. Tarin J, Ten J, Vendrell FJ, de Oliveira MN, Cano A. Effects of maternal ageing and dietary antioxidant supplementation on ovulation, fertilisation and embryo development in vitro in the mouse. Reprod Nutr Dev 1998; 38: 499-508.
297. Tarin JJ, Perez-Albala S, Cano A. Oral antioxidants counteract the negative effects of female aging on oocyte quantity and quality in the mouse. Mol Reprod Dev 2002; 61: 385-97.
298. Murray AA, Molinek MD, Baker SJ, et al. Role of ascorbic acid in promoting follicle integrity and survival in intact mouse ovarian follicles in vitro. Reproduction 2001; 121: 89-96.
299. Tilly JL, Tilly KI. Inhibitors of oxidative stress mimic the ability of follicle-stimulating hormone to suppress apoptosis in cultured rat ovarian follicles. Endocrinology 1995; 136: 242-52.
300. Unfer TC, Conterato GM, da Silva JC, Duarte MM, Emanuelli T. Influence of hormone replacement therapy on blood antioxidant enzymes in menopausal women. Clin Chim Acta 2006; 369: 73-7.
301. Bednarek-Tupikowska G, Tupikowski K, Bidzinska B, et al. Serum lipid peroxides and total antioxidant status in postmenopausal women on hormone replacement therapy. Gynecol Endocrinol 2004; 19: 57-63.
302. Naziroglu M, Simsek M, Simsek H, Aydilek N, Ozcan Z, Atilgan R. The effects of hormone replacement therapy combined with vitamins C and E on antioxidants levels and lipid profiles in postmenopausal women with type 2 diabetes. Clin Chim Acta 2004; 344: 63-71.
303. Ozden S, Dildar K, Kadir YH, Gulizar K. The effects of hormone replacement therapy on lipid peroxidation and antioxidant status. Maturitas 2001; 38: 165-70.