Role of free radicals in female reproductive diseases and assisted reproduction

Dr Ashok Agarwal is the Director of Research at the Centre for Advanced Research in Human Reproduction, Infertility, and Sexual Function, and the Director of the Clinical Andrology Laboratory and Reproductive Tissue Bank. He holds these positions at The Cleveland Clinic Foundation, Ohio, USA, where he is a Professor of Surgery at the Cleveland Clinic Lerner College of Medicine, and since 1993 has been a full staff member in the Glickman Urological Institute, Departments of Obstetrics–Gynecology, Anatomic Pathology, and Immunology. Dr Agarwal has published extensively with over 180 original peer reviewed articles, 20 book chapters, and over 450 presentations at scientific meetings. His research is focused on studies of the role of oxidative stress, DNA integrity, and apoptosis in the pathophysiology of male and female reproduction.

Dr Ashok Agarwal
Ashok Agarwal¹, Shyam SR Allamaneni
Centre for Advanced Research in Human, Reproduction, Infertility, and Sexual Function, Glickman Urological Institute and Department of Obstetrics-Gynecology, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Desk A19.1, Cleveland, OH 44195, USA
¹Correspondence: Fax: +1 216 4456049; e-mail: agarwaa@ccf.org

Abstract
Infertility is a common problem experienced by many couples. Numerous treatments are available for female infertility. However, in some cases, the treatment is empirical in nature because the aetiology of infertility is not fully understood. Recently, reactive oxygen species (ROS) have been shown to have an important role in the normal functioning of reproductive system and in the pathogenesis of infertility in females. Reactive oxygen species may also play a role in other reproductive organ diseases of women such as endometriosis. Oxidative stress develops when there is an imbalance between the generation of ROS and the scavenging capacity of antioxidants in the reproductive tract. It affects both natural and assisted fertility. Because assisted reproductive techniques are used extensively in the treatment of infertility, it is critical to understand the in-vitro conditions that affect fertilization and embryo development. Treatments that reduce oxidative stress may help infertile women with diseases that are caused by this imbalance. Such strategies include identifying the source of excessive generation of ROS, treating the primary cause, and in-vitro and in-vivo supplementation of antioxidants. Research is in progress to identify the mechanisms that are involved in the aetiology of female reproductive diseases caused by ROS, and to create effective strategies that can counteract oxidative stress.

Keywords: antioxidants, embryo, female infertility, free radicals, oxidative stress, reactive oxygen species

Introduction
Successful pregnancy results from an interaction between myriad physiological processes in both men and women. Any disruption to this interactive system, whether in a man or woman, can result in an inability to have a biological child. Infertility can be defined as a lack of pregnancy after 1 year of regular unprotected intercourse. Approximately 15–20% of couples of reproductive age are infertile, which can be attributed equally to both male and female factors.

Reactive oxygen species (ROS) are free radicals implicated in many human diseases. Superoxide anion radical (O₂⁻), hydrogen peroxide (H₂O₂) and the hydroxyl radical (OH*) are the most common type of ROS. When the balance between ROS and antioxidants is tipped towards an overabundance of ROS, oxidative stress occurs. Numerous studies support the role of ROS in male infertility (Agarwal and Saleh, 2002; Saleh and Agarwal, 2002; Aitken et al., 2003). Thus, there is increased interest to examine the role of ROS in female reproduction. Reactive oxygen species have also been implicated in some specific diseases of the female reproductive tract. A comprehensive review that provides information on the role of ROS in female reproductive diseases is lacking. This article discusses how understanding of oxidative stress as a cause of female infertility has evolved and provide information regarding its role in female reproductive pathologies. Treatment strategies to counteract oxidative stress are also presented.

Physiological role of ROS in female reproductive tract
Even though ROS are toxic to human cells, they are normally produced in the body as by products during oxygen...
metabolism. In addition, some cells may have inherent mechanisms that produce ROS for physiological purposes (Halliwell, 1989) such as leukocytes and spermatozoa. Generally, cells express the genes of proteins whose function is important for cell survival and function. Thus, the presence of antioxidant gene expression in the cells of the female reproductive tract indicates that a delicate balance of pro-oxidant/antioxidant exists in the cell environment and that oxidative stress plays a role in the functioning of those cells. Many animal studies have examined the possible role of the ROS–antioxidant balance in oocyte maturation, physiological mechanisms of endometrial shedding have shown that lipids in the membrane until all of them have undergone peroxidative damage. Peroxidation of lipids containing polyunsaturated fatty acids have abundant target sites for ROS because they have double bonds between carbon atoms. The lipid peroxidative process is well studied in spermatozoal membranes (Jones et al., 1979). Peroxidation of lipids in the biological membranes leads to disturbances in the activity of membrane enzymes and ion channels. As a result, the normal cellular mechanisms that are required for fertilization are inhibited. It is possible to measure the extent of peroxidative damage by estimating the stable end products of lipid peroxidation such as malondialdehyde.

Lipid peroxidation is a self-propagating reaction unless it is counteracted by antioxidants. Once ROS acts on membrane lipids, alkyl and peroxy lipid radicals are formed. These radicals, if not quenched by antioxidants, will act on other lipids in the membrane until all of them have undergone peroxidative damage. Embryo development has been negatively associated with elevated concentrations of lipid peroxides (Nasr-Esfahani et al., 1990; Noda et al., 1991).
Figure 1. Mechanisms of oxidative stress-induced cell damage.
Mitochondrial alterations

Reactive oxygen species can affect the mitochondria in oocytes and embryos (Kowaltowski and Vercesi, 1999). Because mitochondrial DNA lack histones, they are prone to oxidative injury. Moreover, mitochondria are one of the main sites for production of ROS. Thus, they are the first cell organelle to be affected. When mitochondria are damaged, ROS can leak into the cytoplasm in increasing amounts (Catt and Henman, 2000). Finally, mitochondria are central to metabolic activities; any disturbances in these activities can lead to profound problems such as ATP generation, which is essential for cellular function.

Peroxidative DNA damage

Deoxyribonucleic acid bases and phosphodiester backbones are also susceptible to peroxidative damage by ROS. Reactions involving ROS and nitrogenous bases or deoxyribose sugars of DNA result in different oxidatively modified products in DNA. The commonly used marker for oxidative DNA damage, 8-hydroxy-2-deoxyguanosine results from reaction between hydroxyl radical and guanine. The presence of these altered nitrogenous bases affects replication and transcription processes and results in mutations and altered gene expressions (Cooke et al., 2003).

Role of apoptosis

Apoptosis is a natural process in which the body removes old and senescent cells; it is a process of programmed cell death. In human germ cells, apoptosis may help remove abnormal germ cells and prevent their overproduction. Multiple extrinsic and intrinsic cell factors control the process of apoptosis. Reactive oxygen species may also initiate a chain of reactions that ultimately lead to apoptosis. This particular function of ROS may be physiological in nature, like in cyclical luteal regression. However, when there is an imbalance between ROS/antioxidants, apoptosis may occur pathologically and damage tissues. In the latter case, luteal regression may occur even in the presence of pregnancy, resulting in a lack of luteal hormonal support. Increased H₂O₂ concentrations in fragmented embryos were associated with high apoptosis levels (Yang et al., 1998).

ROS as a cause of abnormal function in ageing oocytes

The ageing of oocytes is associated with congenital anomalies in children (Tarin et al., 2000). Possibly because ROS injure the oocytes (Tarin, 1996). The senescent process may involve oxidative damage to mitochondrial DNA, proteins and lipids. A decrease in intracellular ATP and glutathione (GSH)/glutathione disulphide (GSSG) ratio and an increase in cytosolic calcium ions have been reported. These changes may harm cytoskeleton fibres and impair fertilization and embryo development, all of which occur more frequently in pregnancies in older women (Tarin, 1996).

Natural protection against ROS

Because ROS have both physiological and pathological functions, the human body has developed defence systems to maintain their concentrations within a certain range. The female reproductive system is rich in both enzymatic and non-enzymatic antioxidants. Catalase, SOD and glutathione peroxidase/reductase are enzymatic antioxidants that prevent ROS from acting on cellular molecules (Li et al., 1993).

Superoxide dismutase is the first enzyme in the defensive cascade. It acts on toxic superoxide radicals and is very important for cell survival. Concentrations of SOD in the rat corpus luteum change during the ovulatory cycle and during pregnancy (Sugino et al., 1996). Multiple non-enzymatic antioxidants are present in the follicular and tubal fluids, and they provide external protection to the gametes and embryo. These antioxidants include vitamin C, vitamin E, glutathione, taurine and hypotaurine. Vitamin C, which act as chain-breaking antioxidant, prevents the propagation of the peroxidative process.

Whenever ROS concentrations become pathologically elevated and oxidative stress occurs, antioxidants begin to work mainly by preventing ROS formation and repairing ROS induced damage. Peroxidation reactions are augmented by metallic ions such as iron. Thus, metal-binding proteins like albumin and transferrin also play a significant role in preventing the propagation of the peroxidative process.

Estimation of oxidative stress

Various methods are available to estimate levels of oxidative stress. Only electron spin resonance method can directly measure ROS concentrations in the body. The most common methods of estimation measure concentrations of a stable peroxidation end product. Chemiluminescence, and flow cytometry measure oxidative end product formed in vitro by interaction of ROS with a reagent. Other methods measure endogenous end products such as lipid peroxidation products (e.g. malondialdehyde and conjugated dienes), oxidized proteins (e.g. carbonyl proteins) and DNA oxidation markers (8-hydroxydeoxyguanosine). Malondialdehyde and other stable end products of lipid peroxidation can be estimated using the thiobarbituric acid assay.

Oxidative stress can also be estimated by measuring concentrations of individual antioxidants or the total antioxidant capacity (TAC) of the system. The enhanced chemiluminescence assay and calorimetric assay techniques are commonly used to measure TAC. The results are expressed as micromolar of Trolox equivalents. Other methods can be used to measure TAC, such as oxygen radical absorbance capacity, ferric reducing ability and the phycoerythrin fluorescence-based assay.

Chemiluminescence assay

The chemiluminescence assay is commonly used to measure concentrations of ROS. Luminol (3-amino-2, 3-dihydro-1, 4-phthalazinedione) and lucigenin (bis-N-methylacridinium nitrate) are used as probes. Luminol measures both intracellular and extracellular ROS including O₂⁻, H₂O₂ and OH⁻ (Sharma and Agarwal, 1996). It provides an overall measurement of ROS in a given sample. The chemical reagents that are involved in the chemiluminescence assay are extremely light sensitive. Either photon counting or current
counting luminometers can be used to measure the luminescence. The results can be expressed as counted photon per minute (cpm), relative light units (RLU) and millivolts per second (mV/s).

Flow cytometry can be used to measure concentrations of ROS in cells. An individual intracellular ROS radical can be identified separately using a low number of cells. 2',7'-Dichlorofluorescein diacetate (DCFH-DA) and hydroethidine (HE) are used to detect \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) respectively. This technique has been used to measure ROS concentrations in embryos (Yang et al., 1998).

**Role of ROS in female infertility**

Understanding of the role of ROS in female infertility is still incomplete. The fact that oxidative and antioxidant systems are present in various female reproductive tissues suggests that infertility and certain reproductive disease such as endometriosis and hydrosalpinx may be caused, at least in part, by oxidative stress (Murphy et al., 1998a;b; Bedaiwy et al., 2002a,b; Agarwal et al., 2003).

**Defects in folliculogenesis and oocyte maturation**

The graffian follicle contains many potential sources of ROS, including large numbers of macrophages, neutrophils and metabolically active granulosa cells. Many studies have found ROS and antioxidants in follicular fluid (Paszkowski et al., 1995, 2002; Jozwik et al., 1999; Attaran et al., 2000; Oyawoye et al., 2003). Follicular fluid contains high concentrations of antioxidants, which protect oocytes from ROS-induced damage. It is possible that an imbalance in pro-oxidant/antioxidant systems in follicular fluid may lead to abnormal development of oocytes and impaired fertility as well as damage to the oocyte’s DNA, cytoskeleton or membrane. The cytoskeleton helps ensure that meiosis occurs in the oocyte, which is a pre-requisite before fertilization for formation of haploid gamete.

A study by Paszkowski et al. demonstrated selenium dependent glutathione peroxidase (GSHPx) activity in the follicular fluid of women undergoing IVF (Paszkowski et al., 1995). The authors reported that follicular selenium concentrations were significantly lower in patients with unexplained infertility compared with those with tubal infertility or couples with male factor infertility. Follicles with oocytes that were subsequently fertilized had higher GSHPx activity than the follicles with oocytes that failed to fertilize. This suggests that oxidative stress is present in the oocyte environment in infertile women and that a lack of antioxidant activity negatively affects the fertilizability of the oocyte.

Jozwik et al. reported the presence of oxidative stress markers (conjugated dienes, lipid hydroperoxides and thiobarbituric acid-reactive substances) in the follicular fluid and serum of patients undergoing IVF (Jozwik et al., 1999). The concentrations of these oxidative stress markers were lower in the follicular fluid than the serum, indicating follicular fluid contains high concentrations of antioxidant systems, which help protect an oocyte from oxidative damage. These authors failed to find any relationship between oxidative stress markers and pregnancy outcome; however, pregnancy outcome in an IVF programme could be affected by many other variables.

A study by Attaran et al. demonstrated that ROS concentrations in follicular fluid were positively associated with pregnancy in patients undergoing IVF (Attaran et al., 2000). It is possible that the ROS concentrations in this patient population represent normal physiological concentrations of ROS in follicular fluid. This finding also emphasizes the need for establishing a reference ROS value in follicular fluid.

A recent study measured the role of non-enzymatic antioxidants in follicular fluid of women undergoing IVF (Oyawoye et al., 2003). The authors found that baseline TAC concentrations were higher in follicles whose oocytes fertilized successfully. However, TAC concentrations were lower in the follicles that produced embryos that were capable of surviving transfer.

**Oxidative stress in peritoneal cavity**

Peritoneal fluid is critical for normal fertilization and early embryonic development. According to one study, the peritoneal fluid in women with endometriosis and unexplained infertility negatively affected sperm function (Oak et al., 1985). It is possible that ROS and toxic peroxidation products such as lipid peroxides are responsible for this negative effect. Peritoneal fluid contains a number of cells that can produce ROS such as macrophages. Normally, antioxidants in peritoneal fluid keep ROS concentrations within a physiological range. Elevated concentrations of ROS can damage the ovum after its release from the ovary, the zygote/embryo and, most importantly, spermatozoa. It is now well known that a minimal amount of ROS is required for the sperm–oocyte fusion process, but excessive concentrations are detrimental to the spermatozoa (Saleh and Agarwal, 2002).

Elevated concentrations of ROS in peritoneal fluid may partially be to blame for unexplained infertility. Wang et al. compared ROS concentrations in peritoneal fluid between women undergoing laparoscopy for infertility evaluation and fertile women undergoing tubal ligation. The authors found higher ROS concentrations in the patients with idiopathic infertility in both unprocessed and processed (after cell separation) peritoneal fluid that reached statistical significance in processed peritoneal fluid ROS concentrations (Wang et al., 1997). Another study also reported a significant difference in ROS concentrations in unprocessed peritoneal fluid between women with idiopathic infertility and healthy fertile women (Bedaiwy et al., 2002). These results suggest that ROS may play a role in patients with idiopathic infertility. Estimating ROS concentrations in the peritoneal fluid may help to identify the cause of infertility in cases of so-called idiopathic infertility.

Polak et al. studied the concentrations of antioxidants in patients with unexplained infertility, tubal infertility, endometriosis and fertile women with benign, non-inflamatory ovarian tumours. The authors found that concentrations of antioxidants in the peritoneal fluid of patients with unexplained infertility were significantly lower than concentrations in the fertile patients (0.49 ± 0.21 versus 0.67...
embryos in Fallopian tubes could be damaged as well. In continuous contact with the peritoneal fluid, early stage
damage to oocytes and spermatozoa. Because tubal fluid is
in infertile women, which may result in oxidative stress
These results indicate that the pro-oxidant/antioxidant balance is
malondialdehyde in the peritoneal fluid than the fertile women.
Women with idiopathic infertility had higher concentrations of
oxidative stress in endometriosis and infertility associated with
endometriosis is needed. Further research regarding the exact role of
of oxidatively damaged molecules may lead to the identification
of persistent markers of oxidative stress (end products
between healthy women and those with endometriosis (Wang
Two studies failed to detect a difference in ROS concentrations
between healthy women and those with endometriosis (Wang et
These authors suggested that elevated ROS concentrations may be a local phenomenon occurring at the site of disease and may not be detectable in peritoneal fluid (Van Langendonckt et al., 2002). Therefore, the estimation of persistent markers of oxidative stress (end products of oxidatively damaged molecules) may lead to the identification of oxidative stress in the peritoneal environment (Bedaiwy and Falcone, 2003). Further research regarding the exact role of oxidative stress in endometriosis and infertility associated with endometriosis is needed.

ROS as a mediator of Fallopian tube pathologies
Hydrosalpinx is accumulation of toxic fluid in the Fallopian tubes in conditions that causes an inflammatory reaction. One study demonstrated the presence of ROS, antioxidants and lipid peroxidation products in hydrosalpingeal fluid (SF) (Bedaiwy et al., 2002b). In this study, the mouse embryo blastocyst development rate was positively related to increasing ROS concentrations in HSF ($P < 0.02$). The low concentrations of ROS detected in HSF in this study may not represent toxic levels. Rather, it could be a marker for normal tubal secretory function.

Oxidative stress and embryo development
The effect of oxidative stress on early embryonic development is an area of intense research. Assisted reproductive techniques provide an in-vitro model to study the factors that affect embryo development. Under in-vitro conditions, only a few oocytes develop into good quality embryos; the rest show abnormal morphology due to unequal cell division and fragmentation (Goyanes et al., 1990). In-vitro culture conditions such as a high oxygen concentration may be the cause of defective embryo development. Enough evidence is available to suggest that embryos produce ROS, which may originate from embryo metabolism and from the surrounding environment (Nasr-Esfahani et al., 1992; Goto et al., 1993). Reactive oxygen species act on the cellular molecules of the embryo and may block or retard early embryonic development (Guerin et al., 2001).

Sources of ROS in embryos
Reactive oxygen species may originate internally from embryo cells or from the external environment. A preimplantation embryo can generate ROS, which involves oxidative phosphorylation, NADPH oxidase and xanthine oxidase (Filler and Lew, 1981).

Exogenous sources of ROS appear to be important for assisted reproduction. Concentrations of ROS are higher in embryos cultured under in vitro conditions than in those cultured in in vivo conditions (Goto et al., 1993). Various exogenous environmental conditions may influence ROS concentrations, including oxygen concentration, metallic ions, visible light and amine oxidases from dead spermatozoa, the latter of which catalyzes spermine and spermidine into hydrogen peroxide and other products. The oxygen concentration in the Fallopian tubes is lower than the atmospheric oxygen concentration. Production of ROS increases under atmospheric oxygen concentration in mouse embryos (Goto et al., 1993). Enhanced embryo development in the mouse is associated with a reduction in oxygen concentration.

Antioxidants protection of embryo against ROS
Embryos use many mechanisms to protect themselves from the oxidative stress inside itself and that from the surrounding environment (Paszkowski and Clarke, 1996; Guядer-Joly et al., 1998). The environment surrounding the oocyte and embryo contains non-enzymatic antioxidants such as vitamin C, glutathione, hypotaurine and taurine, which protect the embryo from external sources of ROS. Similarly, multiple internal antioxidants are available for protection against ROS, like SOD, catalase and glutathione peroxidase (Li et al., 1993).
Effects of ROS on embryos

Reactive oxygen species retard embryo development by affecting the key cellular organelle required for rapid division of cells. In addition following events may occur: aggregation of cytoskeleton components, condensation of the endoplasmic reticulum and loss of membrane fluidity. Embryo cleavage depends partly on microtubules and membrane fluidity; any disturbances in these factors can arrest embryo development (Catt and Henman, 2000).

Apoptosis is another process by which ROS affect embryos. In one study, hydrogen peroxide concentrations were significantly higher in fragmented embryos than in non-fragmented embryos (72.21 versus 31.30). Apoptosis was seen only in the fragmented embryos, indicating that high concentrations of hydrogen peroxide may cause embryo fragmentation (Yang et al., 1998).

Clinical significance of ROS estimation in culture media

A recent study examined the role of ROS in embryo development (Bedaiwy et al., 2004). In this study, ROS concentrations in day 1 culture media showed an inverse correlation with fertilization ($P = 0.037$) and blastocyst development ($P = 0.027$) in patients undergoing ICSI. High concentrations of ROS in day 1 culture media were associated with lower pregnancy rates for both IVF ($P = 0.01$) and ICSI ($P = 0.002$) cycles. It was concluded that ROS concentrations in day 1 culture media might help predict fertilization, embryo development and pregnancy. Unlike previous studies, this study showed that ROS affects not only fertilization and embryo development, but also the real outcome for a patient, in terms of the clinical pregnancy rate.

Oxidative stress and assisted reproduction

There may be multiple sources of ROS in an IVF setting, including the oocytes (4–5 per dish), cumulus cell mass (thousands of cells) and spermatozoa used for insemination ($150 \times 10^3–200 \times 10^3$ per dish) (Bedaiwy et al., 2004). The potential cellular sources of ROS in an ICSI setting consist of the oocytes and the injected spermatozoa. Because oocytes are denuded of cumulus cells, these do not contribute to the ROS generation during ICSI.

The greatest source of exogenous ROS appears to be spermatozoa that are used to inseminate oocytes in vitro, but other sources cannot be ruled out, especially since ROS is known to affect the ICSI, where single spermatozoa is used. Long insemination times (14-16 h) used in IVF laboratories may prolong the exposure of oocytes to spermatozoa and increase the chances of oxidative damage. Therefore, a number of laboratories are investigating ways to reduce exposure time. The results have been conflicting. Investigations with shorter insemination times of gametes have shown beneficial effects in IVF (Gianaroli et al., 1996; Quinn et al., 1998), but some reports found no difference (Boone and Johnson, 2001).

Management of oxidative stress in female reproductive tract

Even though there is no definitive consensus on the use of antioxidants in patients with infertility, many in-vitro and in-vivo studies have shown that they improve semen quality and fertility in men (Agarwal et al., 2004). Although few studies have focused on the role of antioxidants in female infertility, it is anticipated that they may improve fertility in women as well. A recent randomized controlled, multi-centre study evaluated the effect of vitamin C supplementation (750 mg/day) in patients with a luteal phase defect (Henmi et al., 2003). The authors reported that the pregnancy rate was significantly higher in the treatment group compared with the controls [25% (19/76) versus 11% (5/46)]. The authors also reported that serum progesterone concentrations were significantly elevated in the treatment group. The basis for these results is supported by a study in which concentrations of antioxidants substances were found to be significantly lower in women with a history of recurrent miscarriages and luteal phase defects than in healthy women (Vural et al., 2000). Another study reported that vitamin C concentrations were higher in the follicular fluid of patients supplemented with vitamin C than that of the controls (Crha et al., 2003). Those authors also found that the pregnancy rate was higher in the supplemented group than in the control group (34.2 versus 23.7%), but the difference was not statistically significant.

It is currently unknown which type of antioxidants, if any, should be used to help treat female infertility. Dose and duration of treatment is a subject requiring further study. It should be noted that at higher concentrations, antioxidants such as vitamin C may act as pro-oxidants. Vitamin C acts as reducing agent at higher concentrations, and in higher doses was postulated to be a cause of infertility (Briggs, 1973). Recent studies in animals have reported that although administration of oral antioxidants can counteract the negative effects of female ageing on number and quality of oocytes (Tarin et al., 2002a), they may induce side effects on reproductive fitness and impair the ovarian and uterine functions of females (Tarin et al., 2002b).

Once oxidative stress is diagnosed, treatment plans must focus on identifying and eliminating the source of ROS. In most cases, oxidative stress appears to be a result of increased generation of ROS rather than a depletion of antioxidants. When a specific cause is identified, medical and surgical management options should be considered to eliminate the source of ROS. Unlike in men, specific clinical conditions associated with oxidative stress have yet to be identified in women. This complicates the treatment of the primary cause of excessive ROS production. Only after treatment of the primary aetiology, should patients be advised to take antioxidant supplementation. Antioxidants could be started directly, however, when a specific aetiology cannot be identified (idiopathic infertility), as there is no other evidence-based treatment for idiopathic infertility in women and reports have indicated the presence of oxidative stress in these patients. Because a history of smoking is associated with high concentrations of oxidative stress (Paszkowski et al., 2002), in-vivo antioxidants can be recommended to infertile women who smoke.
Management of oxidative stress in assisted reproduction conditions

It is important to avoid the conditions that promote ROS generation and exposing gametes and embryos to ROS during assisted reproduction. One approach may consist of measuring the in-vivo concentrations of oxygen in the oviduct and uterus and simulating similar conditions in vitro. The fertilization and embryo development in vitro appears to take place in an environment of low oxygen tension (Burton et al., 2003). When the oxygen tension decreased in the gas phase during culture (6% CO₂/5% O₂/89% N₂), the implantation and pregnancy rate significantly improved compared with higher oxygen tension (5% CO₂ in air) (Catt and Henman, 2000).

One source of ROS in assisted reproduction is inseminated spermatozoa. Sperm preparation is necessary to enhance and maintain sperm quality and function after ejaculation and before the semen specimen is used for ART (Alvarez, 2003). A proper sperm preparation method must be selected to minimize production of ROS in the seminal fluid. Sperm preparation techniques that separate mature spermatozoa and thus minimize the interaction between ROS producing cells in semen (e.g., leukocytes, immature abnormal spermatozoa) and normal spermatozoa can be used. Density gradient separation and the swim-up method are good sperm preparation methods available currently. It is also possible to avoid ROS from spermatozoa by minimizing the length of time that the oocytes are exposed to spermatozoa.

ROS and in-vitro media

In the absence of ROS quenching antioxidants, it is possible that ROS accumulate in the culture medium and damage spermatozoa, oocytes and embryos. Adding antioxidants to assisted reproduction media may help prevent oxidative injury to oocytes and embryos. The type and concentrations of antioxidants to be used in these media are still under intense investigation. Most of the available studies have used animal models. In-vitro experiments have shown that adding antioxidants to the in-vitro media helps prevent ROS-induced damage and preserves the quality of spermatozoa and embryos. A recent study has demonstrated that the adding antioxidants, especially vitamin C, can improve the blastocyst development rate in a mouse embryo model (Wang et al., 2002).

The authors of a controlled study in an IVF programme found that the implantation and clinical pregnancy rates were higher when antioxidant-supplemented media was used rather than standard media without antioxidants (Catt and Henman, 2000). It is also important to note that interactions between antioxidants and other substances in the media such as metal ions can sometimes result in the production of oxidants. Metal ions can also increase the production of ROS directly. It may be useful to add metal ion chelating agents to the culture media to decrease the production of oxidants.

Discussion on available literature

Published studies vary in their conclusions about the role oxidative stress plays in female reproduction for a number of reasons. Although it is clear that ROS have both a physiological function and pathological role in the female reproductive tract, reference values for ROS (minimum safe concentrations or physiologically beneficial concentrations) have yet to be defined. In addition, the study populations are not uniform. For example, studies performed to assess the role of ROS in a group of IVF patients may produce confounding factors because the indication for IVF in some patients may be some pathology that is unrelated to ROS. Therefore, patients should be separated according to the aetiological factor and analysed separately.

The measurement of oxidative stress in biological systems is a subject of controversy. Many types of methods are available. A uniform method or related methods should be used so that the results can be compared across the studies. Comparing results from studies that used different measures of oxidative stress may lead to incorrect/erroneous conclusions. For example, measurement of ROS concentrations and peroxidative end products such as lipid peroxides is not comparable across studies because ROS concentrations indicate oxidative stress at the point of measurement, whereas peroxidative end products may represent oxidative stress over a period of time depending on the half-life. For example, studies may measure only an individual antioxidant or TAC.

The outcome parameters (fertilization, embryo development, and pregnancy) used as endpoints should also be similar across studies so that the results can be more easily compared. In addition, many other factors can influence the outcome at any point in time from fertilization to pregnancy, especially the conditions of the embryo culture when assisted reproduction is used. Thus, an attempt should be made by researchers to identify if any other confounding factors are present between study groups when comparing the results.

Summary of possible mechanisms of oxidative stress–induced infertility in women

(i) Direct damage to oocytes due to oxidative stress in ovarian follicles. (ii) Direct damage to oocytes and spermatozoa due to oxidative stress in the peritoneal cavity. (iii) Direct damage to embryo due to oxidative stress in the Fallopian tube. (iv) ROS–antioxidant imbalance leading to defective endometrium for supporting embryo. (v) ROS–antioxidant imbalance leading to luteal regression and lack of luteal hormonal support for continuation of pregnancy.

In cases where there is direct injury to the gametes, the damage may lead to impaired fertilization. Even when fertilization occurs, the embryo fragmentation, implantation failure, abortion or congenital anomalies in offspring can occur depending on degree of damage to gametes. Direct injury to an embryo may immediately lead to embryo fragmentation or affect the future development of the embryo and health of the child.

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Received 9 June 2004; refereed 21 June 2004; accepted 5 July 2004.