
The Impact of Reactive Oxygen Species on Early Human Embryos: A Systematic Review of the Literature

-- Sajal Gupta, Jashoman Banerjee, Ashok Agarwal

Abstract: Oocytes and embryos are constantly exposed to oxidative stress which influences their developmental competence. But, the antioxidants present in the reproductive tissues protect the embryos *in vivo*.

The effects of oxidative stress in Assisted Reproductive Technology (ART) is amplified by lack of physiological defense by the antioxidants and also due to abundance of potential sources that can generate Reactive Oxygen Species (ROS) in culture media. Certain levels of ROS are necessary for sperm function, normal activity in the ovarian follicle, normal sperm-oocyte interaction and sperm capacitation. But, excessive levels may have adverse effects on sperm DNA, fertilization and embryo quality.

ROS not only accelerate apoptosis in the cell by direct DNA damage, but also affect the DNA repair mechanisms along with alterations in important check points in cell cycle. *In-vitro* developmental arrests are documented in mammalian embryos that are exposed to oxidative stress. Hence blastocyst development *in vitro* lags behind blastocyst development *in vivo*. Sperm DNA damage caused by elevated ROS levels results in embryo development arrest and poor fertility outcomes with ART. This article reviews the literature on the sources of ROS generation in ART setting and enumerates strategies to overcome oxidative stress.

Key Words: reactive oxygen species (ROS), oxidative stress, embryos developmental arrest

Infertility is a disease of the reproductive system characterized by inability to conceive and carry a pregnancy to delivery after a year of unprotected intercourse. Infertile couples represent 10%-15% of all couples. Assisted Reproductive Technologies (ART) have become one of the standard treatment options for couples with male factor, endometriosis, tubal disease and unexplained infertility. In spite of advancements in ART, the outcomes remain unsatisfactory.

A majority of the mature oocytes retrieved fertilise *in vitro* but only 70% of these undergo the first three cleavage divisions during the first 3 days in culture, Less than 50% of the cleaved embryos undergo cavitation and proceed to form blastocysts while only 30% of embryos which form on day 3, progress to develop into morphologically normal blastocysts.

To search for an answer, researchers have focused on identifying factors limiting embryonic development in *in-vitro* media.

Postal Address: Department of Obstet-Gynecology, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA

Email Address: agarwaa@ccf.or

Oxidative stress (OS) has been implicated as one of the factors responsible for unsatisfactory outcomes in ART (Agarwal *et al.*, 2005a, 2005,b). The role of OS in the pathogenesis of male infertility has been demonstrated in various studies (Said *et al.*, 2005). Oxidative stress induced sperm damage leads to poor fertility outcomes both *in vivo* and *in vitro* (Agarwal *et al.*, 1994, Agarwal *et al.*, 2003, Saleh *et al.*, 2002 Said *et al.*, 2005.).

All the components of the female reproductive tract, starting with the ovaries to the uterine milieu, are also exposed to oxidative insult. However, the amount of stress which is detrimental has not been not quantified till date (Attaran *et al.*, 2000, Pasqualotto *et al.*, 2004, Bedaiwy 2005).

Oxidative stress and anti-oxidants

Oxidative stress is the result of an imbalance between the unstable metabolites of oxygen, named as reactive oxygen species (ROS) and their normal scavengers, the anti oxidants (Pasqualotto *et al.*, 2004). The ROS can be radicals or non-radicals and the anti oxidants are enzymes or other substances *viz.* catalase, superoxide dismutase, glutathione peroxidase, ascorbate, tocopherol etc. (Sharma *et al.*, 2004).

Some amount of ROS is needed in the ovarian follicle (Attaran *et al.*, 2000) as well as for normal sperm-oocyte interaction and sperm capacitation (de Lamirande *et al.*, 1997). However, raised levels of ROS have a deleterious effect on cell membranes, cellular DNA, mitochondria and ultimately accelerates cell death either by apoptosis or necrosis (Alvarez 2003).

Oxidative stress in ART and embryo development

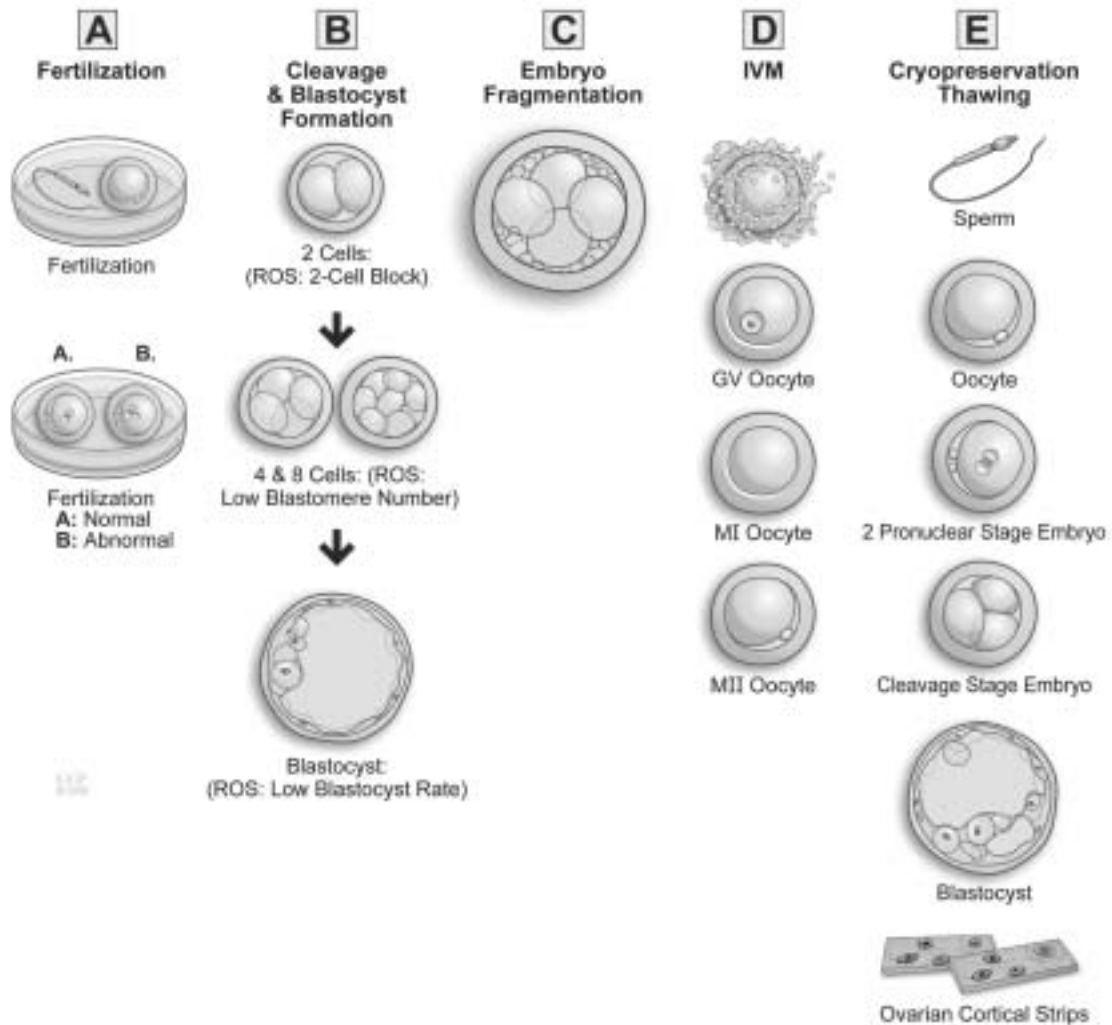
OS can cause defective embryo development and retardation (Agarwal *et al.*, 2003, Guerin *et al.*, 2001) that is attributed to induced cell membrane damage, DNA damage and apoptosis (as shown in figure) Apoptosis results in fragmented embryos, which have limited potential to implant and hence result in poor fertility outcomes (Jurisicova *et al.*, 1996).

Increased OS in the male germ cells has also been associated with poor fertilization rates, impaired embryo development and higher rates of pregnancy loss (Baker *et al.*, 2005)

In an ART set up, the physiological milieu is simulated by modification of the media as well as the technique used. However, an *in vitro* set up can never mimic the exact physiology of an *in-vivo* condition. Embryos cultured *in vitro* are exposed to amplified amounts of oxidative stress. This is due to the lack of antioxidants which act as natural physiological defense against OS as well as the presence of potential sources that can generate ROS in the embryo culture media. The sources of ROS can be internal or external.

The use of antioxidants

The fertilization and embryo development *in vivo* takes place in an environment of low oxygen tension (Burton *et al.*, 2003). Lowering oxygen tension in the culture environment also improves the implantation and pregnancy rate (Catt *et al.*, 2000). Similarly, higher implantation and clinical pregnancy rates are reported when culture media are supplemented with antioxidants.

Possible effects of Oxidative Stress (OS) during the various stages of *in vitro* embryonic development

(A) Fertilisation: Certain levels of ROS are needed for the sperm function and consequently it's fertilizing capacity. (B) Cleavage and blastocyst formation: Embryos produce ROS by oxidative phosphorylation, nicotinamide adenine dinucleotide phosphate, oxidase and xanthine oxidase systems. OS is implicated in low embryonic cell number and low blastocyst development. (C) Embryonic fragmentation: OS is implicated in embryonic fragmentation and apoptosis. (D) Oocyte *in vitro* maturation: Cumulus cells provide pyruvate and/or lactate as oxidative substrates for the oocyte during IVM. Addition of follicular fluid during IVM protects the oocytes from oxidative stress through the radical scavenging activity elicited by SOD isoenzymes. This results in the enhancement of cytoplasmic maturation and developmental competence post-fertilization. (E) Cryopreservation-thawing: Cryopreserved spermatozoa showed ROS induced membrane lipid damage and limited antioxidant defenses. Oocyte cryopreservation may be associated with aberrant patterns of cytokinesis. Such an effect may be extrapolated to the other stages of embryo development. Ovarian tissue cryopreservation using a slow cooling protocol results in significantly elevated ROS levels and apoptosis after warming.

Metal ions also induce production of ROS directly through the Haber-Weiss reaction. Addition of metal ion chelating agents to the culture media may decrease the production of oxidants and help in successful embryo development and pregnancy (Catt *et al.*, 2000).

Addition of ascorbate during cryopreservation has also been reported to reduce the levels of hydrogen peroxide and thus prevent oxidative distress to mammalian embryos (Lane *et al.*, 2002). Thus, antioxidants are likely to play significant role not only during ART procedures but also in preventing subsequent loss or damage to embryo.

Internal Sources of ROS

Energy needs of a developing embryo are high and are met by generating ATP through oxidative phosphorylation and glycolysis (Thompson *et al.*, 2000). Aerobic metabolism is inherently linked to the generation of ROS. This metabolism can generate excess of ROS in the system which when inhibited can give positive results in in-vitro outcomes (Thompson *et al.*, 2000, Machaty Z. 2000). Thus, the embryo itself is a major source of ROS, as oxygen metabolism fulfils its inherent energy needs.

It produces ROS like any actively metabolizing cell in the body. The generation of ROS depends on three enzyme systems *viz.*, oxidative phosphorylation, NADPH oxidase and xanthine oxidase systems (Guerin *et al.*, 2001). There can be other oxidase enzymes involved in the process of generation of ATP and subsequently raised ROS levels. The embryos can generate

O_2^- , H_2O_2 , and OH^- as seen in rabbit blastocysts appearing 4/5 days after coitus (Manes *et al.*, 1995).

The ROS levels have been reported to be raised *in-vitro culture* as compared to *in vivo* in mice (Goto *et al.*, 1993). Different stages of embryo development have varied levels of ROS generation. In mouse embryos ROS is generated twice, at the time of fertilization and the G2/M phase of the second cell cycle (Nasr-Esfahani *et al.*, 1990, Nasr-Esfahani *et al.*, 1991).

It has also been reported that the generation of ROS is not always dependent on the mitochondria. Manes and Lai (1995) demonstrated that cyanide which irreversibly inhibits mitochondrial respiration, did not reduce generation of ROS in rabbit blastocysts. This implies that there are other sources responsible for generation of oxygen radicals apart from that during oxygen metabolism.

Antioxidants play a significant role not only during the ART procedures but also in preventing subsequent loss or damage to embryo.

Another oxidizing system found in the pre-implantation embryo is the NADPH oxidase. The NADPH oxidase system can also produce free radicals as was observed in rabbit blastocysts (Manes *et al.*, 1995). Inhibition of NADPH oxidase system can prevent generation of H_2O_2 in 2-cell mouse embryos (Nasr-Esfahani *et al.*, 1991). Whether a similar system exists in human embryos and whether it is responsible for the developmental arrests of embryos needs to be studied.

Purine metabolism can also contribute to production of ROS. Xanthine has been reported to be the end product of purine

metabolism in the mouse pre-implantation embryo development (Alexiou *et al.*, 1992) and can arrest development of mouse embryos *in vitro* (Nureddin *et al.*, 1990). The metabolism of purines during the early phases of embryogenesis is high and in the process, increased levels of ROS can be generated. During or just before the genomic activation, the activity of adenosine deaminase is very high and so is the purine salvaging activity of various enzymes. This purine salvaging can limit ROS generation. This relates to the activity of an enzyme hypoxanthine phosphoribosyl transferase (HPRT) that prevents the accelerated catabolism of purines due to increased mRNA inactivation (Guerin *et al.*, 2001).

The contribution of some other oxidase systems have also been implicated, but their role seems species specific. One such is glycolate oxidase, detected in mouse embryos (Khatchadourian *et al.*, 1994).

External sources of ROS

The embryos are one of the sources of ROS in an ART setting but they also bear the brunt of insult from the ROS generated externally in the media and environment. The external sources are plenty and the technique of ART itself also contributes to the generation of ROS. The external factors may be oxygen concentration, visible light, amine oxidase, media and additives, spermatozoa, excess glucose, metallic ions, freeze-thaw process, and many pollutants etc.

Oxygen concentration: It is well known that an *in-vitro* system has more oxygen tension than an *in-vivo* system. It is been observed that the Fallopian tubal cells have one-third of the atmospheric oxygen tension (Mastroianni *et al.*, 1965, Maas *et al.*, 1976). The physiological concentration of oxygen in the cells is approx. 10 μ M/L, whereas in the media at room temperature it is nearly 224 μ M /L. It has been known that higher oxygen tension above 5% had detrimental effects on development of fertilized mouse oocytes (Eppig *et al.*, 1995).

Gaseous
environment of the
incubators influence
generation of
oxidative stress.
Reduction of oxygen
concentration improves
in-vitro development
of embryos.

This high oxygen tension activates various oxidase enzyme systems in the cells and helps generate radicals within them. The lower tension *in vivo* protects the cells from pro oxidant insult. The reduction of oxygen tension *in vitro* resulted in better embryo development in mice (Goto *et al.*, 1993), reverse 2-cell block (Pabon *et al.*, 1989) and improve murine blastocyst cell numbers (Orsi *et al.*, 2001).

Gaseous environment of the incubators can influence generation of oxidative stress indirectly by altering gaseous properties and oxygen tension. One report states that incubation in gaseous mixtures of 5% CO₂, 5% O₂ 90% N₂ produced more blastocysts than those incubated under 5% CO₂ in air. This influence of the gaseous environment is more in *in vitro* embryos than the *in vivo* ones (Booth *et al.*, 2005). Reduction of O₂ conc. from 20% to 5% demonstrated improved *in-vitro* development of embryos (Thompson *et al.*, 1990, Watson *et al.*, 1994, Quinn *et al.*, 1978)

Metallic ions: Metallic ions such as iron (Fe) and copper (Cu) can accelerate ROS generation within the cell by participating in *Fenton* and *Haber-Weiss* equations. These ions when present in the media may get incorporated in the cells during the various processing techniques. The adverse effects of metallic ions can be reversed by addition of metal chelators in the media. Addition of EDTA (ethylenediamine tetraacetic acid) or transferrin overcomes such developmental arrests (Orsi *et al.*, 2001, Nasr-Esfahani *et al.*, 1992)

Visible light: Cell membranes have unsaturated lipids and cholesterols. Visible light induces photodynamic stress and can cause oxidative damage to these lipids (Girotti 2001) which generate ROS that damage DNA. (Beehler *et al.*, 1992). Literature emphasizes that duration for which the embryos are exposed to light is vital for such ROS generation. Some researchers believe that even transient exposure can affect the embryo (Nakayama *et al.*, 1994) while others report that > 5 min of exposure to light is sufficient to cause a major increase in H₂O₂ in mouse embryos (Goto *et al.*, 1993)

Spermatozoa: Spermatozoa act as carriers of ROS since they lack any scavenging system. The spermatozoa affected by oxidative stress can not only affect the oocyte after penetration, but the spermatozoa that remain outside induce oxidative stress in the media. Hence the number of sperm exposed to oxidative stress is important (Alvarez *et al.*, 1996). Duration of spermatozoa oocyte contact is significant factor

that governs generation of ROS. Spermatozoa that have already been damaged due to oxidative stress may induce changes in the oocyte because it depends on the oocyte for the DNA repair process (Alvarez 2003). Hence tools to detect damage or apoptotic spermatozoa before IVF is vital (Said *et al.*, 2006). Dead spermatozoa liberate amine oxidase (Shannon 1978) which react with spermine and spermidine to produce hydrogen peroxide and other amine compounds (Parchment *et al.*, 1990). Short insemination times can thus reduce the chances of the

spermatozoa death and subsequent generation of amine oxidase in the media (Quinn *et al.*, 1998).

Human oocytes have a narrow optimal insemination window (Ermilov *et al.*, 1999). Studies also show that in-vitro insemination in mice when delayed by 2 hours reduces blastocyst development (Edgar *et al.*, 1987).

Sperm preparation techniques that remove the spermatozoa affected by oxidative stress as well as the apoptotic spermatozoa should be used.

Appropriate timing of insemination and shortening the exposure of oocyte to spermatozoa may be important modalities in reducing oxidative stress levels in the ART setting.

Media and additives: In an ART setting specific commercial IVF media can generate ROS depending on their composition and subsequently affect the quality of the oocyte

Shortening the duration of exposure of oocyte to spermatozoa may be important modalities in reducing oxidative stress levels in the ART setting.

and the embryo. Simple media reduce such incidences. Commonly used additives in the media such as serum contain high levels of amine oxidase that may actually increase the oxidant load in the media and accelerate ROS production. Protein supplementation of the media leads to reduced apoptosis level and enhances hatching rates in mouse embryos .

To reduce the generation of ROS due to external sources means revising culture protocols with reference to the choice of media, the additives to the media; decrease the oocyte-handling time, decrease the exposure to media that generates more ROS.

ROS induced damage

Studies have been conducted in animal and human systems to locate the origin of ROS as well as use various antioxidants to obtain better fertility outcomes (Guerin *et al.*, 2001, Choi *et al.*, 2005) . Effect of ROS on sperm DNA has been established and also correlated with poor ART outcomes (Saleh *et al.*, 2003, Sharma *et al.*, 2004, Agarwal *et al.*, 2005). Poor embryo quality in cases of ART using sperm with abnormal DNA (Sakkas *et al.*, 1998, Agarwal *et al.*, 2005) and raised apoptosis have been documented (Aitken *et al.*, 2001, Twigg *et al.*, 1998). ROS can not only accelerate apoptosis in the cell by direct DNA damage, but also affect the DNA repair mechanisms along with alterations in important check points in cell cycle (Barzilai *et al.*, 2004).

Embryo development and effect of oxidative stress have been thoroughly studied both in animals and human systems. Differential growth patterns have been correlated to ROS levels on day 1 to day 7 in human embryo

cultures (Bedaiwy *et al.*, 2004). This study demonstrated increased embryonic fragmentation and lower cleavage rates in intra cytoplasmic sperm injection cycles with raised day 1 ROS. The pathogenesis can be stretched back to the basic energy requirement of embryos. Raised ROS can inactivate glyceraldehydes 3 phosphate dehydrogenase and thus reduce ATP generation (Halliwell 1989). Embryo development arrests are also related to embryos originating from damaged DNA in the spermatozoa used for ART. Cellular mitochondrial damage also decrease the ATP content (Taanman 1999) and oxidative stress can raise the mitochondrial DNA disruption, due to lack of histones (Kowaltowski *et al.*, 1999, Richter *et al.*, 1995). This implies increased insult to embryo development in culture media.

In-vitro developmental arrests are documented in mammalian embryos that are exposed to oxidative stress (Johnson *et al.*, 1994). Mouse embryos demonstrated a 2-cell block with raised ROS only after culture whereas this effect was not found in embryos retracted *in-vivo* (Noda *et al.*, 1994). Even it is suggested that improper oocyte maturation may reflect as abnormal embryo development (Blondin *et al.*, 1997). Hence in an *in-vitro* set up the insult of oxidative stress seem to get amplified.

Many animal studies have indicated a detrimental response in embryo development due to effect of oxidative stress in early stages (Nasr-Esfahani *et al.*, 1990, Goto *et al.*, 1993) either due to increased generation of ROS or lack of natural anti oxidant defenses like taurine, hypotaurine, glutathione which exists in oviduct and uterine milieu (Gardiner *et al.*, 1998, Guerin *et al.*, 1995, Gardiner *et al.*, 1994).

Evidence also suggests that later stages of embryo development like the morula and blastocyst show varied effects due to generation of oxidative stress in the media as well as in the number of dividing cells. Analysis of affected embryos demonstrated a reduction in glutathione content, accelerated apoptosis involving the caspase 3 system and increased membrane lesions in the inner cell mass (ICM) (Feugang *et al.*, 2003).

Strategies to overcome OS affecting embryos in ART setting

Strategies to overcome oxidative stress include both *in vivo* and *in-vitro* antioxidant supplementation. Literature support that antioxidant supplementation can result in generation of better quality embryos. Supplementation of the media with optimal concentration of antioxidants, amino acids and vitamins C, E results in scavenging of the free radicals. Supplementation of culture media with antioxidants; disulphide reducing agents or divalent chelators of cations is beneficial for embryos cultured in vitro. Enhanced embryo survival and blastulation rates have been reported with antioxidant supplementation of the media. Pentoxifylline, an antioxidant and immunomodulator significantly reduces the embryotoxic effects of hydrogen peroxide on mouse 2 cell embryos (Zhang *et al.*, 2005).

Antioxidants such as Ethylene diaminetetraacetic acid (EDTA), low oxygen tension, superoxide dismutase can be utilized in overcoming OS in the ART setting

Antioxidants are used as media supplements for various sperm preparation techniques. Patients with elevated ROS in semen can benefit from density gradient centrifugation and glass wool filtration to scavenge ROS. Pentoxifylline, glutathione and albumin are effective in reducing ROS levels, when used in sperm preparation media. Sperm preparation helps reduce the exposure of the functional spermatozoa to the defective spermatozoa and leucocytes.

Two randomized controlled trials reported reducing sperm-oocyte interaction times resulted in production of better quality embryos and increased implantation and pregnancy rates (Gianaroli *et al.*, 1996, Kattera *et al.*, 2003). Optimizing techniques which lead to reducing generation of OS in the ART setup will help improve fertility outcomes.

Enhanced embryo survival and blastulation rates have been reported with antioxidant supplementation of the media.

REFERENCES

Agarwal A and Said TM (2005) Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. *BJU Int* 95, 503-7.

Agarwal A, Allamaneni SS, Nallella KP, George AT and Mascha E (2005a) Correlation of reactive oxygen species levels with the fertilization rate after in vitro fertilization: a qualified meta-analysis. *Fertil Steril* 84, 228-31.

Agarwal A, Gupta S and Sharma R (2005b) Oxidative stress and its implications in female infertility - a clinician's perspective. *Reprod Biomed Online* 11, 641-50.

Agarwal A, Gupta S and Sharma RK (2005c) Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol* 3, 28.

Agarwal A, Ikemoto I and Loughlin KR (1994) Relationship of sperm parameters with levels of reactive oxygen species in

semen specimens. *J Urol* 152, 107-10.

Agarwal A, Saleh RA and Bedaiwy MA (2003) Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 79, 829-43.

Aitken RJ and Krausz C (2001) Oxidative stress, DNA damage and the Y chromosome. *Reproduction* 122, 497-506.

Alexiou M and Leese HJ (1992) Purine utilisation, de novo synthesis and degradation in mouse preimplantation embryos. *Development* 114, 185-92.

Alvarez JG (2003) DNA fragmentation in human spermatozoa: significance in the diagnosis and treatment of infertility. *Minerva Ginecol* 55, 233-9.

Alvarez JG, Minaretzis D, Barrett CB, Mortola JF and Thompson IE (1996) The sperm stress test: a novel test that predicts pregnancy in assisted reproductive technologies. *Fertil Steril* 65, 400-5.

Attaran M, Pasqualotto E, Falcone T, Goldberg JM, Miller KF, Agarwal A and Sharma RK (2000) The effect of follicular fluid reactive oxygen species on the outcome of in vitro fertilization. *Int J Fertil Womens Med* 45, 314-20.

Baker MA and Aitken RJ (2005) Reactive oxygen species in spermatozoa: methods for monitoring and significance for the origins of genetic disease and infertility. *Reprod Biol Endocrinol* 3, 67.

Barzilai A and Yamamoto K (2004) DNA damage responses to oxidative stress. *DNA Repair (Amst)* 3, 1109-15.

Bedaiwy MA, Agarwal A., Falcone T., Goldberg JM., Arrigain S., Mascha E. (2005) Relationship of follicular fluid oxidative stress parameters and outcome of intracytoplasmic sperm injection. *Fertility and sterility, ASRM abstracts* 84., S250.

Bedaiwy MA, Falcone T, Mohamed MS, Aleem AA, Sharma RK, Worley SE, Thornton J and Agarwal A (2004) Differential growth of human embryos in vitro: role of reactive oxygen species. *Fertil Steril* 82, 593-600.

Beehler BC, Przybyszewski J, Box HB and Kulesz-Martin MF (1992) Formation of 8-hydroxydeoxyguanosine within DNA

of mouse keratinocytes exposed in culture to UVB and H₂O₂. *Carcinogenesis* 13, 2003-7.

Blondin P, Coenen K and Sirard MA (1997) The impact of reactive oxygen species on bovine sperm fertilizing ability and oocyte maturation. *J Androl* 18, 454-60.

Booth PJ, Holm P and Callesen H (2005) The effect of oxygen tension on porcine embryonic development is dependent on embryo type. *Theriogenology* 63, 2040-52.

Burton GJ, Hempstock J and Jauniaux E (2003) Oxygen, early embryonic metabolism and free radical-mediated embryopathies. *Reprod Biomed Online* 6, 84-96.

Catt JW and Henman M (2000) Toxic effects of oxygen on human embryo development. *Hum Reprod* 15 Suppl 2, 199.

Choi W, Banerjee J, Agarwal A, Falcone T and Sharma R (2005) Can Vitamin C Supplementation Reduce Oxidative Stress Induced Cytoskeleton Damage of Mouse Oocyte. *Fertility and sterility, ASRM abstracts* 84, S452.

de Lamirande E, Leclerc P and Gagnon C (1997) Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization. *Mol Hum Reprod* 3, 175-94.

Edgar DH, Whalley KM and Mills JA (1987) Preimplantation development following in vitro fertilization of mouse oocytes: effects of timing of superovulation and preincubation in vitro. *J In Vitro Fert Embryo Transf* 4, 111-5.

Eppig JJ and Wigglesworth K (1995) Factors affecting the developmental competence of mouse oocytes grown in vitro: oxygen concentration. *Mol Reprod Dev* 42, 447-56.

Ermilov A, Diamond MP, Sacco AG and Dozortsev DD (1999) Culture media and their components differ in their ability to scavenge reactive oxygen species in the plasmid relaxation assay. *Fertil Steril* 72, 154-7.

Esfandiari N, Falcone T, Agarwal A, Attaran M, Nelson DR and Sharma RK (2005) Protein supplementation and the incidence of apoptosis and oxidative stress in mouse embryos. *Obstet Gynecol* 105, 653-60.

Feugang JM, Van Langendonck A, Sayoud H, Rees JF, Pampfer

- S, Moens A, Dessy F and Donnay I (2003) Effect of prooxidant agents added at the morula/blastocyst stage on bovine embryo development, cell death and glutathione content. *Zygote* 11, 107-18.
- Gardiner CS and Reed DJ (1994) Status of glutathione during oxidant-induced oxidative stress in the preimplantation mouse embryo. *Biol Reprod* 51, 1307-14.
- Gardiner CS, Salmen JJ, Brandt CJ and Stover SK (1998) Glutathione is present in reproductive tract secretions and improves development of mouse embryos after chemically induced glutathione depletion. *Biol Reprod* 59, 431-6.
- Gianaroli L, Fiorentino A, Magli MC, Ferraretti AP and Montanaro N (1996) Prolonged sperm-oocyte exposure and high sperm concentration affect human embryo viability and pregnancy rate. *Hum Reprod* 11, 2507-11.
- Girotti AW (2001) Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. *J Photochem Photobiol B* 63, 103-13.
- Goto Y, Noda Y, Mori T and Nakano M (1993) Increased generation of reactive oxygen species in embryos cultured in vitro. *Free Radic Biol Med* 15, 69-75.
- Guerin P and Menezo Y (1995) Hypotaurine and taurine in gamete and embryo environments: de novo synthesis via the cysteine sulfinic acid pathway in oviduct cells. *Zygote* 3, 333-43.
- Guerin P, El Mouatassim S and Menezo Y (2001) Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Hum Reprod Update* 7, 175-89.
- Halliwell B (1989) Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. *Br J Exp Pathol* 70, 737-57.
- Johnson MH and Nasr-Esfahani MH (1994) Radical solutions and cultural problems: could free oxygen radicals be responsible for the impaired development of preimplantation mammalian embryos in vitro? *Bioessays* 16, 31-8.
- Juriscova A, Varmuza S and Casper RF (1996) Programmed cell death and human embryo fragmentation. *Mol Hum Reprod* 2, 93-8.
- Kattera S and Chen C (2003) Short coincubation of gametes in in vitro fertilization improves implantation and pregnancy rates: a prospective, randomized, controlled study. *Fertil Steril* 80, 1017-21.
- Khatchadourian C, Guillaud J and Menezo Y (1994) Interactions in glycine and methionine uptake, conversion and incorporation into proteins in the preimplantation mouse embryo. *Zygote* 2, 301-6.
- Kowaltowski AJ and Vercesi AE (1999) Mitochondrial damage induced by conditions of oxidative stress. *Free Radic Biol Med* 26, 463-71.
- Lane M, Maybach JM and Gardner DK (2002) Addition of ascorbate during cryopreservation stimulates subsequent embryo development. *Hum Reprod* 17, 2686-93.
- Maas DH, Storey BT and Mastroianni L, Jr. (1976) Oxygen tension in the oviduct of the rhesus monkey (*Macaca mulatta*). *Fertil Steril* 27, 1312-7.
- Machaty Z, ALR, Thompson J.G., Day B.N., Prather R.S. (2000) Inhibition of Oxidative Phosphorylation and its Effect on Porcine Embryonic Development. *Theriogenology* 53, 277.
- Manes C and Lai NC (1995) Nonmitochondrial oxygen utilization by rabbit blastocysts and surface production of superoxide radicals. *J Reprod Fertil* 104, 69-75.
- Mastroianni L, Jr. and Jones R (1965) Oxygen Tension within the Rabbit Fallopian Tube. *J Reprod Fertil* 9, 99-102.
- Nasr-Esfahani MH, Aitken JR and Johnson MH (1990) Hydrogen peroxide levels in mouse oocytes and early cleavage stage embryos developed in vitro or in vivo. *Development* 109, 501-7.
- Nasr-Esfahani MH, Winston NJ and Johnson MH (1992) Effects of glucose, glutamine, ethylenediaminetetraacetic acid and oxygen tension on the concentration of reactive oxygen species and on development of the mouse preimplantation embryo in vitro. *J Reprod Fertil* 96, 219-31.

- Nasr-Esfahani MM and Johnson MH (1991) The origin of reactive oxygen species in mouse embryos cultured in vitro. *Development* 113, 551-60.
- Noda Y, Goto Y, Umaoka Y, Shiotani M, Nakayama T and Mori T (1994) Culture of human embryos in alpha modification of Eagle's medium under low oxygen tension and low illumination. *Fertil Steril* 62, 1022-7.
- Nureddin A, Epsaro E and Kiessling AA (1990) Purines inhibit the development of mouse embryos in vitro. *J Reprod Fertil* 90, 455-64.
- Orsi NM and Leese HJ (2001) Protection against reactive oxygen species during mouse preimplantation embryo development: role of EDTA, oxygen tension, catalase, superoxide dismutase and pyruvate. *Mol Reprod Dev* 59, 44-53.
- Pabon JE, Jr., Findley WE and Gibbons WE (1989) The toxic effect of short exposures to the atmospheric oxygen concentration on early mouse embryonic development. *Fertil Steril* 51, 896-900.
- Parchment RE, Lewellyn A, Swartzendruber D and Pierce GB (1990) Serum amine oxidase activity contributes to crisis in mouse embryo cell lines. *Proc Natl Acad Sci U S A* 87, 4340-4.
- Pasqualotto EB, Agarwal A, Sharma RK, Izzo VM, Pinotti JA, Joshi NJ and Rose BI (2004) Effect of oxidative stress in follicular fluid on the outcome of assisted reproductive procedures. *Fertil Steril* 81, 973-6.
- Quinn P and Harlow GM (1978) The effect of oxygen on the development of preimplantation mouse embryos in vitro. *J Exp Zool* 206, 73-80.
- Quinn P, Lydic ML, Ho M, Bastuba M, Hendee F and Brody SA (1998) Confirmation of the beneficial effects of brief coincubation of gametes in human in vitro fertilization. *Fertil Steril* 69, 399-402.
- Richter C, Gogvadze V, Laffranchi R, Schlapbach R, Schweizer M, Suter M, Walter P and Yaffee M (1995) Oxidants in mitochondria: from physiology to diseases. *Biochim Biophys Acta* 1271, 67-74.
- Said T, Agarwal A, Grunewald S, Rasch M, Baumann T, Kriegel C, Li L, Glander HJ, Thomas AJ, Jr. and Paasch U (2006) Selection of nonapoptotic spermatozoa as a new tool for enhancing assisted reproduction outcomes: an in vitro model. *Biol Reprod* 74, 530-7.
- Said TM, Aziz N, Sharma RK, Lewis-Jones I, Thomas AJ, Jr. and Agarwal A (2005) Novel association between sperm deformity index and oxidative stress-induced DNA damage in infertile male patients. *Asian J Androl* 7, 121-6.
- Sakkas D, Urner F, Bizzaro D, Manicardi G, Bianchi PG, Shoukir Y and Campana A (1998) Sperm nuclear DNA damage and altered chromatin structure: effect on fertilization and embryo development. *Hum Reprod* 13 Suppl 4, 11-9.
- Saleh RA and Agarwal A (2002) Oxidative stress and male infertility: from research bench to clinical practice. *J Androl* 23, 737-52.
- Saleh RA, Agarwal A, Nada EA, El-Tonsy MH, Sharma RK, Meyer A, Nelson DR and Thomas AJ (2003) Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. *Fertil Steril* 79 Suppl 3, 1597-605.
- Shannon P (1978) Factors affecting semen preservation and conception rates in cattle. *J Reprod Fertil* 54, 519-27.
- Sharma RK and Agarwal A (2004) Role of reactive oxygen species in gynecologic diseases. *Reprod Med Bio* 3, 177-199.
- Sharma RK, Said T and Agarwal A (2004) Sperm DNA damage and its clinical relevance in assessing reproductive outcome. *Asian J Androl* 6, 139-48.
- Taanman JW (1999) The mitochondrial genome: structure, transcription, translation and replication. *Biochim Biophys Acta* 1410, 103-23.
- Thompson JG, McNaughton C, Gasparini B, McGowan LT and Tervit HR (2000) Effect of inhibitors and uncouplers of oxidative phosphorylation during compaction and blastulation of bovine embryos cultured in vitro. *J Reprod Fertil* 118, 47-55.

Thompson JG, Simpson AC, Pugh PA, Donnelly PE and Tervit HR (1990) Effect of oxygen concentration on in-vitro development of preimplantation sheep and cattle embryos. *J Reprod Fertil* 89, 573-8.

Twigg JP, Irvine DS and Aitken RJ (1998) Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. *Hum Reprod* 13, 1864-71.

Watson AJ, Watson PH, Warnes D, Walker SK, Armstrong DT and Seamark RF (1994) Preimplantation development of in vitro-matured and in vitro-fertilized ovine zygotes: comparison between coculture on oviduct epithelial cell monolayers and culture under low oxygen atmosphere. *Biol Reprod* 50, 715-24.

Zhang X, Sharma RK, Agarwal A and Falcone T (2005) Effect of pentoxifylline in reducing oxidative stress-induced embryotoxicity. *J Assist Reprod Genet* 22, 415-7.

Dr. Ashok Agarwal is the Director of Research at the Center 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, where he is a Professor in the Cleveland Clinic's Lerner College of Medicine of Case Western Reserve University and, since 1993, a senior staff in the Glickman Urological Institute, Departments of Obstetrics-Gynecology, Anatomic Pathology, and Immunology. Dr. Agarwal has published extensively with over 240 original peer reviewed articles, 20 book chapters, and over 550 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.