ANTIOXIDANTS, NUTRITION AND FERTILITY

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ABSTRACT

Oxidative stress is a state related to an increased cellular damage produced by oxygen and oxygen derived free radicals known as reactive oxygen species (ROS). It is a serious condition, as ROS and their metabolites attack DNA, lipids and proteins, alter enzymatic systems and cell signalizing pathways producing irreparable alterations, cell death and necrosis. While small amounts of ROS have been shown to be required for some physiological functions, their excessive levels can impair an overall fertilization capacity. This questions have recently attracted the attention of the scientific society, however, research in exploring the role of oxidative stress and antioxidants associated with fertility remains in his initial stages and therefore needs further exploration. This review summarizes the latest consensus on the role of ROS and antioxidants in reproduction. It should not only help to clarify the role of ROS and antioxidants in reproduction, but also explain the impact of antioxidant supplementation via nutrition on an overall fertility.

Keywords: oxidative stress, reactive oxygen species, antioxidants, fertility, nutrition

INTRODUCTION

Reproductive failure is a significant public health concern. Infertility, defined as the failure to conceive a recognized pregnancy after 12 months of unprotected intercourse, carries significant personal, societal and financial consequences **(Goldman et al., 2000)**. Approximately 15%-20% of couples of reproductive age are infertile, which can be attributed equally to both male and female factors **(Agarwal, Allamaneni, 2004)**.

Although relatively little is known about factors affecting fertility and early pregnancy loss, there is sufficient evidence to hypothesize that dietary antioxidants and oxidative stress (OS) may influence the timing and maintenance of a viable pregnancy **(Ruder et al., 2008)**.

Oxidative energy production is inevitably associated with the generation of reactive oxygen species (ROS), excessive concentrations of which can lead to cellular pathology. Germ cells and embryos are as vulnerable as other cells to the potential detrimental effects of ROS and may thus require antioxidant protection at sites of gamete production, maturation and storage and embryo implantation. In addition, attention has focused on a possible role for antioxidant therapy to alleviate infertility. The two decades since the first *in vitro* fertilization success **(Steptoe et al., 1980)** have witnessed an explosion in infertility treatments, which has led to the development of new and improved techniques as both adjunct and alternative to invasive treatments, and provided material for research **(Taylor, 2001)**.

Inevitably, as these methods require manipulation of gametes and embryos *in vitro*, these cells may be exposed to additional oxidative stress necessitating exogenous protection. Moreover recent work has shown ROS to have signaling capabilities and there is emerging evidence that crucial reproductive processes may be influenced or mediated by ROS. There is thus considerable interest in the roles of antioxidants and ROS in infertility **(Ford, 2001)**.

FREE RADICALS AND REACTIVE OXYGEN SPECIES

Under physiological conditions, biomolecules are comprised of stable bonds formed by paired electrons. Weakened, disrupted bonds allow for the generation of free radicals **(Pierce et al., 2004)** - short lived, unstable and highly reactive chemical intermediates, which contain one or more unpaired electrons **(Sanocka, Kurpisz, 2004)**. They induce cellular damages when they pass this unpaired electron onto nearby cellular structures, resulting in oxidation of cell membrane lipids, amino acids in proteins or within nucleic acids **(Agarwal et al., 2004)**.

Reactive oxygen species are formed endogenously during aerobic metabolism and as a result of various metabolic pathways of the cell or as part of the body's defense mechanisms. ROS also can arise from exogenous sources, such as alcohol, tobacco, and various environmental pollutants, such as heavy metal contamination **(Agarwal et al., 2008b; Tvrda et al., 2010a)**.

ROS represent a broad category of molecules that indicate the collection of radicals (hydroxyl ion, superoxide, nitric oxide, peroxyl, etc.) and nonradicals (ozone, single oxygen, lipid peroxides, hydrogen peroxide) and oxygen derivatives **(Agarwal, Prabakaran, 2005)**. Reactive nitrogen species (nitrous oxide, peroxynitrite, nitroxyl ion, etc.) are free nitrogen radicals and considered a subclass of ROS **(Sikka, 2001)**.

ROS are characterized by their ability to react with any molecule they come in contact and modify it oxidatively. The modification may result in structural and functional alterations and impair many cellular processes. Depending on their tissue concentration they can either exert beneficial physiologic effects (e.g. play role in fertilization process) or pathological damage to cellular components, including lipids, proteins and nucleic acids **(Sharma, Agarwal, 1996)**.

Sources of ROS

Leukocytes and immature spermatozoa are the two main sources of ROS in males **(Garrido et al., 2004)**. Leukocytes particularly neutrophils and macrophages have been associated with excessive ROS production and they ultimately cause sperm dysfunction **(Ford, 2001)**. The fluctuations in ROS levels might be due to transient subclinical infection and transient abnormalities in spermatogenesis such as, retention of cytoplasm or periodic presence of abnormal spermatozoa in semen **(Desai et al.,**

2010).
Also, several biomarkers indicative of redox status have been identified within the ovary, endometrium, fallopian tubes, embryo, placenta, and the peritoneal fluid **(Agarwal et al., 2005)**. The sources of ROS in Graafian follicle may be macrophages, neutrophils and granulosa cells **(Capcarova et al., 2009)**.
Two of the main factors contributing to ROS accumulation in vitro are the absence of endogenous defense mechanism and second exposure of gametes and embryos to various manipulation techniques as well as environment that can lead to generation of oxidative stress. Levels of ROS may rarely fluctuate within a fertile individual, but do not affect sex cell viability. This may be due to the presence of adequate antioxidant defense mechanisms in the healthy individuals **(Shiotani et al., 1991)**.

Positive effects of ROS

The production of ROS is a normal physiological process **(Sharma, Agarwal, 1996)**. According to **Agarwal et al. (2008b)** and **Goncalves et al. (2010)** ROS generated by spermatozoa play an important role in normal physiological processes such as, sperm capacitation, acrosome reaction, maintenance of fertilizing ability, and stabilization of the mitochondrial capsule in the mid-piece of spermatozoa. Controlled generation of ROS may function as signaling molecules (second messengers) in many different cell types; they are important mediators of sperm functions. Evidences have been reported that especially superoxide anion ($O_2^{\bullet}$) is required for the late stage of embryo development such as, two germ cell layers and egg cylinder **(Kodama et al., 1996).** Although a significant negative correlation between ROS and *in vitro* fertilization (IVF) rate has been found **(Agarwal et al., 2005)**, yet, controlled generation of ROS has shown to be essential for the development of capacitation and hyperactivation **(De Lamirande, Cagnon, 1993)**; the two processes of sperm that are necessary to ensure fertilization. *In vivo* physiological concentrations of ROS are involved in providing membrane fluidity, maintaining the fertilizing ability and acrosome reaction of sperm **(Bucak et al., 2010)**. The maintenance of a suitable ROS level is, therefore, essential for adequate sperm functionality.
Many studies reported the presence of oxidative and antioxidant systems in various female reproductive tissues **(Bedaiwy et al., 2002)**. ROS appears to have physiological role in female reproductive tract in many different processes such as: oocyte maturation, fertilization, luteal regression, and endometrial shedding **(Sugino et al., 2004)**. ROS levels in follicular fluid may be used as markers for predicting the success of *in vitro* fertilization **(Agarwal et al., 2003)**.

OXIDATIVE STRESS AND INFERTILITY

The term Oxidative stress (OS) is generally applied when oxidants outnumber antioxidants **(Sies, 1993)**, when peroxidation products develop **(Spiteller, 1993)** and when these phenomena cause pathological effects **(Janssen et al., 1993)**. The imbalance between the production of ROS and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage is known as OS **(Agarwal et al., 2003)**. It is a serious condition causing toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA **(Schafer, Buettner, 2001)**. The effects of OS depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis **(Lennon et al., 1991)**. The assumption that ROS can influence male fertility has received substantial scientific support **(Agarwal et al., 2005)**.

Lipid peroxidation

Lipids are considered to be the most susceptible macromolecules and are present in sperm plasma membrane in the form of polyunsaturated fatty acids (PUFA); fatty acids that contain more than two carbon-carbon double bonds. These fatty acids maintain the membrane fluidity **(Agarwal, Saleh, 2002)**. ROS attacks PUFA leading to a cascade of chemical reactions called lipid peroxidation. Peroxidation of PUFA results in the loss of membrane fluidity and a reduction in the activity of membrane enzymes and ion channels. As a consequence, normal cellular mechanisms that are required for fertilization are inhibited **(Agarwal, Allamamneni, 2006**). Lipid peroxidation in spermatozoa is a self-propagating reaction unless counteracted by seminal antioxidants. Once ROS acts on membrane lipids, alkyl and peroxyl 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 **(Agarwal, Saleh, 2002)**.

DNA damage

DNA bases and phosphodiester backbones are other sides that are susceptible to peroxidative damage by ROS. High levels of ROS mediate the DNA fragmentation that is commonly observed in the spermatozoa of infertile individuals **(Kodama et al., 1997; Sun et al., 1997)**. Normally, sperm DNA is protected from oxidative insult by its specific compact organization and by antioxidants in the seminal plasma. Spermatozoa are unique in that they cannot repair DNA and depend on the oocyte for repair after fertilization **(Aitken et al., 2003)**. Various types of DNA abnormalities occur in sperm that have been exposed to ROS artificially. These abnormalities include base modifications, production of base-free sites, deletions, frame shifts, DNA cross-links and chromosomal rearrangements **(Agarwal, Said, 2003; Duru et al., 2000)**. OS is also associated with high frequencies of single and double strand DNA breaks **(Duru et al., 2000; Aitken, Krausz, 2001)**. ROS can also cause gene mutations such as point mutation and polymorphism, resulting in decreased semen quality **(Spiropoulos et al., 2002)**. Other mechanisms such as denaturation and DNA base-pair oxidation may also be involved **(Kodama et al., 1997)**. DNA damage in the Y chromosome can also cause gene deletion in the Y chromosome of the offspring leading to infertility **(Aitken, Krausz, 2001)**.

Oxidative damage to proteins

Oxidative attack on proteins results in site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis. The amino acids in a peptide differ in their susceptibility to attack, and the various forms of activated oxygen differ in their potential reactivity. Primary, secondary and tertiary protein structures alter the relative susceptibility of certain amino acids. Sulfur containing amino acids and thiol groups specifically, are very susceptible **(Farr, Kogama, 1991)**.

Oxidative stress and apoptosis

ROS may also initiate a chain of reactions that ultimately lead to apoptosis. Apoptosis is a natural process in which the body removes old and senescent cells; it is a process of programmed cell death. Apoptosis may help remove abnormal germ cells and prevent their overproduction **(Agarwal, Allamaneni, 2006)** and helps in elimination of abnormal spermatozoa. High levels of ROS disrupt the inner and outer mitochondrial membranes, releasing the cytochrome-c protein and activating the caspases and apoptosis. Apoptosis in sperm cells may also be initiated by ROS-independent pathways involving the cell surface protein Fas **(Sakkas et al., 1999)**.

Effects of OS on spermatozoa motility

Increased ROS levels have been correlated with decreased sperm motility **(Armstrong et al., 1999)**. However, the exact mechanism through which this occurs is not understood. One hypothesis suggests that H_2O_2 diffuses across the membranes into the cells and inhibits the activity of some vital enzymes **(Aitken, 1997)**. Another involves a series of interrelated events resulting in a decrease in axonemal protein phosphorylation and sperm immobilization, both of which are associated with a reduction in membrane fluidity that is necessary for sperm-oocyte fusion **(De Lamirande, Gagnon, 1992)**. Loss of motility observed when spermatozoa are incubated overnight is highly correlated with lipid peroxidation status of the spermatozoa **(Gomez et al., 1998)**. Furthermore, the ability of antioxidants to revive sperm motility is evidence that lipid peroxidation is a major cause for mobility loss in spermatozoa **(Agarwal et al., 2006)**.

Impairment of sperm-oocyte fusion

A minimal amount of ROS is required for the normal sperm-oocyte fusion. Spermatozoa and oocyte has an inbuilt mechanism to prevent excessive production of ROS at the time of sperm-oocyte fusion, this may be by the release of superoxide dismutase, SOD **(Maiorino, Ursini, 2002)**. If there is an abnormality in the production of SOD, ROS generation can continue uninterruptedly and damage both spermatozoa and oocyte. The affect of ROS on sperm fertilizing capacity cannot be quantified by measuring routine semen parameters. It is possible that the levels of ROS needed to impair sperm-oocyte fusion events are lower than those required to affect sperm motility. The inability of sperm to fuse with an oocyte appears to be due to the effects of ROS on the sperm membrane. The lipid peroxidation process results in loss of membrane fluidity due to disorganization of membrane architecture and reduction in the activity of membrane enzymes and ion channels. As a result, spermatozoa are unable to initiate the necessary biochemical reactions associated with acrosome reaction, zona pellucida binding and oocyte penetration **(Aitken et al., 1991; Griveau, Le Lannou, 1997)**.

Oxidants and female fertility

The role of oxidative stress in female reproductive diseases and infertility is under intense investigations. However, oxidative stress can affect the female fertility potential in number of ways. It may affect the ovulation, fertilization, embryo development and implantation **(Agarwal, Allamaneni, 2004)**.

Follicular fluid contains high levels of antioxidants, which protect oocytes from ROS-induced damage. Significantly lower selenium levels were detected in follicular fluid of patients with unexplained infertility compared with those with tubal infertility or couples with male factor infertility **(Paszkowski et al., 1995)**. Another study reported that baseline TAC levels were higher in follicles whose oocytes fertilized successfully **(Oyawoye et al., 2003)**.

Elevated levels of ROS in peritoneal fluid may be the cause of infertility in some women who do not have any other obvious cause. Elevated levels can damage the ovum after its release from the ovary, the zygote/embryo and most importantly, spermatozoa. As discussed previously, spermatozoa are very sensitive to oxidative stress. Studies have compared ROS levels in peritoneal fluid between women undergoing laparoscopy for infertility evaluation and fertile women undergoing tubal ligation. ROS levels in the peritoneal fluid were significantly higher in the patients with idiopathic infertility compared with the fertile women **(Bedawy et al., 2002; Wang et al., 1997)**. High levels of malondialdehyde and low levels of antioxidants in the peritoneal fluid were reported in patients with unexplained infertility compared to controls **(Polak et al., 2001)**.

Effects of oxidants on embryo growth

Oxidative stress appears to have a detrimental effect on the development of embryo. ROS may originate from embryo metabolism and from the surrounding environment **(Tvrda et al., 2010b)**. ROS not only alters most types of cellular molecules but also induces early embryonic developmental block and retardation. According to **Yang et al. (1998)**, high levels of ROS and apoptosis were reported in fragmented embryos compared to non-fragmented embryos. Multiple mechanisms of embryo protection against ROS exist **(Paszkowski et al., 1999)**.

Effects of oxidants on *in vitro* reproduction

DNA damage induced by oxidative stress has important clinical implications in the context of assisted reproduction (ART). Spermatozoa selected for ART most likely originate from an environment experiencing OS, and a large percentage of these sperm may have damaged DNA **(Saleh, Agarwal, 2002)**. There is a strong possibility that spermatozoa with damaged DNA may be used during ART **(Agarwal, Said, 2003)**, which can negatively affect the ART success rate and increase the risk of spontaneous

abortion or offspring with genetic disorders. ROS levels in mature spermatozoa correlate significantly with the fertilizing potential of spermatozoa **(Sukcharoen et al., 1995; Zorn et al., 2003)**. Estimating ROS levels may help predict the success rate of assisted reproduction procedures.

Low levels of ROS play a beneficial role in IVF. Lipid peroxidation (LPO) and total antioxidant capacity (TAC) levels in follicular fluid were found to correlate positively with the pregnancy rates in patients undergoing IVF **(Murphy et al., 1998)**. Minimal levels of ROS are essential and are indicative of metabolic activity within the follicle. High ROS levels in the culture media, however, on the morning after oocyte retrieval correlated with lower blastocyst development, poor fertilization and cleavage rates, and higher embryonic fragmentation following ICSI **(Wu et al., 2003)**. As high levels of ROS have deleterious effects on both the oocyte and the embryo quality in patients with endometriosis, various strategies are designed to minimize the exposure of gametes to environments that lead to elevated free radical generation. Mechanical removal of ROS, and the rinsing of cumulus oophorus during ART, could overcome the deleterious effects of high ROS levels. Hence, there is scientific evidence in the literature that minimal levels of ROS may be necessary for ART, but excess of ROS can lead to poor outcomes **(Jackson et al., 2005)**.

ANTIOXIDANTS

Because ROS have both physiological and pathological functions, the organism developed defense systems to maintain their levels within a certain range. Whenever ROS levels become pathologically elevated, antioxidants begin to work and help minimize the oxidative damage, repair it, or prevent it all together **(Agarwal, Allamaneni, 2006)**.

Antioxidants, in general, are compounds and reactions that dispose, scavenge, suppress the formation of ROS, or oppose their actions **(Sikka et al., 1995)**. According to **Agarwal et al. (2004)**, antioxidants can protect cells against OS via three mechanisms: prevention, interception and reparative method.

Antioxidants can be divided into two main categories:

- Enzymatic,
- Non-enzymatic.

Enzymatic antioxidants

Enzymatic antioxidants are also known as natural antioxidants; they neutralize excess ROS and prevent it from damaging the cellular structure. Enzymatic antioxidants are composed of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), which causes reduction of hydrogen peroxides to water and alcohol **(Agarwal et al., 2008a)**. SOD spontaneously dismutases superoxide anion ($O_2^{-\bullet}$) to form O_2 and H_2O_2 while catalase converts H_2O_2 to O_2 and H_2O. SOD protects cells against spontaneous O_2 toxicity and lipid peroxidation. SOD and catalase also remove $O_2^{-\bullet}$ generated by NADPH oxidase in neutrophils and play a major role in decreasing LPO and protecting spermatozoa against oxidative damage **(Sikka, 2001)**. Catalase presence has been demonstrated for ram and cattle and it has a potential role in ageing process and control of oxidative stress in cells, mainly resulting from H_2O_2 **(Bucak et al., 2007)**.

Nonenzymatic antioxidants

Nonenzymatic antioxidants are also known as synthetic antioxidants or dietary supplements **(Nozkova et al., 2009)**. The body's complex antioxidant system is influenced by dietary intake of antioxidants, vitamins, and minerals such as vitamin C, vitamin E, zinc, taurine, hypotaurine, and glutathione **(Agarwal et al., 2008a)**.

Glutathione (GSH) is a molecule found in a number of cells, able to react with many ROS directly. GSH is also a cofactor for GSHPx that catlyzes the reduction of toxic H2O2 and other hydroperoxides, protecting the mammalian cells from oxidative stress **(Bucak et al., 2007)**. Glutamine has been provided a cryoprotective effect by improving postthaw motility, membrane integrity, and catalase enzyme activity in ram semen **(Bucak et al., 2009)**.

Supplementation of inositol in the extender can improve the motility of frozen thawed bull sperm. Inositol has cryoprotective and antioxidative properties resulting in higher antioxidant GSH activity, acrosome integrity, and intact morphological rates **(Bucak et al., 2010)**.

Cysteine has been shown to penetrate the cell membrane easily, enhancing the intracellular GSH biosynthesis both in vivo and in vitro and protecting the membrane lipids and proteins due to indirect radical scavenging properties **(Hendin et al., 1999)**. It has cryoprotective effect on the functional integrity of axosome and mitochondria improving postthawed sperm motility. It has been proved that thiols such as glutathione and cysteine prevented the loss of sperm motility in frozen thawed bull semen. Cysteine has been shown to prevent the loss in motility of frozen thawed bull, ram, and goat semen and to improve viability, the chromatin structure, and membrane integrity of boar sperm during liquid preservation **(Uysal, Bucak, 2007)**. Cysteine has also improved the porcine oocytes maturation and fertilization *in vitro* **(Aitken et al., 1997)**.

Trehalose or taurine, acts as nonenzymatic scavenger that plays an important role in the protection of spermatozoa against ROS, in case of exposure to aerobic conditions and the freezing—thawing process **(Reddy et al., 2010)**.

A nonpermanent disaccharide has a protective action related both to osmotic effect and specific interactions with membrane phospholipids, rendering hypertonic media, causing cellular osmotic dehydration before freezing and then decreasing the amount of cell injury by its crystallization. Trehalose performs better cryoprotection postthaw fertilizing ability in ram, bull, and mouse sperm due to diminished death and damage of sperm **(Bucak et al., 2007)**.

Bovine serum albumin (BSA) is known to eliminate free radicals generated by oxidative stress and protect membrane integrity of sperm cells from heat shock during freezing-thawing of canine semen **(Uysal, Bucak, 2007).**

Carotenoids such as beta-carotene and lycopene are also important components of antioxidant defense. Beta carotenes protect the plasma membrane against lipid peroxidation in Japanese quails **(Jung et al., 2009)**.

Since long it has been known that supplementation of

culture media with antioxidants such as, ROS scavengers, disulfide reducing, or divalent chelators prolongs the motility of reactivated bull spermatozoa after freezing and thawing. It has been suggested that antioxidant therapy appears to be efficient not only *in vitro* but also *in vivo* **(Tarin et al., 1997)**.

ANTIOXIDANT SUPPLEMENTATION AND FERTILITY

The supplemental intake of vitamins A (the retinoids and carotenoids) vitamin E and/or vitamin C has been demonstrated to be an efficient strategy to improve reproductive function in laboratory and farm animals **(Luck et al., 1995)**. In the mouse, dietary supplementation with vitamins C and E may help prevent the age-associated decrease in ovulation rate after exogenous ovarian stimulation **(Tarin et al., 1998)**.

The case for oral supplementation in humans is more speculative and depends on whether spermatozoa in the male or female tract are exposed to sufficient oxidative stress to overcome their defense mechanisms and whether administration of oral antioxidants can increase antioxidant levels in the reproductive tract and spermatozoa themselves **(Ford, Whittington, 1998)**.

Oral antioxidants may improve sperm quality in heavy smokers **(Dawson et al., 1992)** and in male factor infertility patients **(Lenzi et al., 1993)**. They may also increase fertilization potential in both healthy men with high levels of ROS in semen **(Kessopoulou et al., 1995)** and fertile normospermic men with low fertilization rates in previous IVF cycles **(Geva et al., 1996)**.

However, there remains a debate as to the effectiveness of oral administration of antioxidants as therapy for male subfertility. **Rolf et al. (1999)** treated patients in a prospective double blind study with either vitamins C and E or placebo daily for 8 weeks. However, no changes in semen parameters and no pregnancies were observed. The lack of effect in this study may be related to small sample size, the dose or combination of vitamins used or the choice of patients. Vitamins C and E may protect sperm from oxidative damage given separately but can induce it in combination **(Hughes et al., 1998)** as vitamin C can act as a prooxidant in certain circumstances **(Podmore et al., 1998)** by reducing Fe^{3+} to Fe^{2+} triggering the Fenton reaction. There is also some uncertainty as to whether oral administration of vitamin E increases the concentration in seminal plasma **(Ford, Whittington, 1998)**. The percentage of motile sperm is certainly related to sperm vitamin E concentration, so vitamin E incorporation into sperm may occur, although no relationship has been demonstrated between the total vitamin E concentration in the seminal plasma and the concentration in individual sperm.

There are few data relating to oral antioxidant therapy in the female. As in the male, the question of whether oral antioxidants reach target sites such as the ovarian follicle tube is crucial. However, toxic substances including nicotine metabolites can do so **(Rosevear et al., 1992)** so it is not unreasonable to suppose that there may be circumstances in which follicular defenses are overwhelmed and antioxidant therapy may compensate, although, the optimal drugs, and doses are unknown. Likewise, it is not known whether oral antioxidants would penetrate the tubal or uterine environments either to increase concentrations in the fluids or become incorporated into embryonic, tubal or uterine cell membranes.

Moreover, the safety dosage of oral antioxidants in the reproductive context is unknown. High doses of retinoids may have embryotoxic and teratogenic effects, including neural crest or tube, musculoskeletal and urogenital anomalies **(Hathcock, 1997)**. Large doses of ascorbate may be associated with inhibition of ovarian steroidogenesis **(Levine, Morita, 1985)**. Although ROS are clearly associated with infertility, the case for antioxidant therapy remains debatable, with immense scope for investigation into identification of those patients for whom therapy may be appropriate.

ANTIOXIDANT DIET FOR FERTILITY?

The main role of antioxidants is in protecting cells from free radicals. Since, all of the organs, fluids, and players in the reproductive system are made up of cells, which need protecting, logically, healthy cells means healthy fertility. The antioxidant rich foods and antioxidant supplements ultimately can have an impact on the cell health. One area to be nourished and protected, are the sex cells. The reproduction cycle offers opportunities for the gametes to be impacted by toxins or nutrients. Antioxidants can provide protection from these free radicals so the cell structure is intact and the overall conception is healthy.

Special attention in antioxidants supplementation via nutrition deserve:

- Vitamin E: laboratory experiments have shown that vitamin E was able to extend the life of the common type of human cell. This could have a positive effect on both egg and sperm health by protecting them from damage. Vitamin E has also been shown to prevent miscarriage in mice. Many people do supplement with vitamin E but usually only consume alpha tocopherol, there are at least three others that should be included in the E supplementation (tocotrienols and mixed tocopherols). Vitamin E is found in cereal brans, especially in rice, wheat and oat bran **(Dimitrov et al., 1996)**.
- Lipoic Acid helps to reactivate all of the other antioxidants and rejuvenates itself. It also turns bad genes "off" and boosts glutathione, which is critical for liver detoxification from toxins and excess hormones. Lipoic Acid can be found in spinach, potatoes, and red meats **(Whiteman et al., 1996)**.
- CoQ10 is involved in production of ATP so a lack of this antioxidant could cause a shortage of energy to every system in the body, including the reproductive system. A study in Fertility and Sterility shared that CoQ10 helped to increase the sperm motility in semen. CoQ10 is found in salmon and meats **(Bahar et al., 2010)**.
- Vitamin C is easily accepted into the cells to provide protection from free radicals. Vitamin C has been shown to help protect sperm from free radical damage. Humans are one of the few organisms who does not produce their own vitamin C, therefore, it has to come from diet or

supplementation. Vitamin C is found in many fruits and vegetables including red peppers, cranberries, broccoli, cabbage, tomatoes, potatoes, and citrus fruits **(Naidu, 2003)**.

- Iron: iron-rich food (lean meat and poultry) in the diet or iron supplements are essential to improve fertility. Researchers from the Harvard School of Public Health studied the diet information of 18,500 pre-menopausal women who conceived or attempted to conceive over an eight-year period. They found that women who used iron supplements were 40% less likely to have infertility problems related to ovulation than those who did not use iron supplements **(Brown et al., 2007)**.
- Calcium and Vitamin D: calcium is necessary for proper hormone balance while low levels of vitamin D have been linked to infertility. Therefore, it is important to eat low-fat dairy products and have lots of natural sunlight **(Farmakiotis, 2007)**.
- Magnesium: together with calcium, magnesium can help support the rhythmic contractions of the fallopian tubes, which in turn help sperm to meet the egg for conception. Magnesium can be found in brown rice, nuts, barely, legumes and green leafy vegetables **(Ford et al., 2007)**.

By far the largest published systematic screening of antioxidants in fresh fruits, vegetables and processed items is a study titled based on measurements of the total antioxidant concentration of 1113 food samples obtained from the US Department of Agriculture National Food and Nutrient Analysis Program **(Halvorsen et al., 2006)**.

- Among 22 ranked food groups, these had the highest antioxidant contents, in the following category order: (1) spices and herbs; (2) nuts and seeds; (3) chocolates and sweets; (4) vegetables; (5) ready-to-eat cereals; (6) desserts and cakes; (7) berries; (8) fruits; (9) beverages; (10) soups, sauces, gravies and dressings; (11) fast foods.
- Grains and dairy products had only modest concentrations.
- Several food categories contained products almost devoid of antioxidants: fats and oils; meat, poultry, seafood and eggs.
- Of the 50 food products highest in antioxidant concentrations, 13 were spices, 8 were fruits and vegetables, 5 were berries, 5 were chocolate-based, 5 were breakfast cereals, and 4 were nuts or seeds.
- On the basis of typical serving sizes, blackberries, walnuts, strawberries, artichokes, cranberries, brewed coffee, raspberries, pecans, blueberries, ground cloves, grape juice, and unsweetened baking chocolate were at the top of the ranked list.

According to **Halvorsen et al. (2006)** together with **Ninfali et al. (2006)**, some fertility „superfoods" recommended for a pre-conceptual diet are:

- Citrus fruits with a high vitamin C content which has been shown to improve fertility levels,
- Sunflower seeds and nuts because of the zinc content.
- Brown rice, oat, pulses and green vegetables for B vitamins. Vitamin B6 is particularly important as it helps to metabolize hormones like estrogen, which could assist in getting pregnant.
- Oily fish, linseed or flaxseeds for omega 3 fatty acids.
- Water and herb or fruit teas.

Foods to avoid when trying to get pregnant:

- Alcohol and caffeine, which inhibit vitamin absorption.
- Sugar, which can depress the immune system.
- Highly processed foods such as microwave dinners. They are often very high in salt and low in essential vitamins. The microwaving process also destroys vitamins.

CONCLUSION

Free radicals and oxidative stress have important roles in modulating many physiological functions in reproduction, as well as in conditions such as infertility, abortion, fetal embryopathies and pregnancy complications. The evaluation of in vivo oxidative stress is difficult. The minimum well tolerated concentration, and the need to be defined. Longitudinal studies to assess various biomarkers during pregnancy may help identify their association with congenital fetal malformations or pregnancy complications. There is emphasis on overcoming oxidative stress during artificial insemination and *in vitro* techniques **(Agarwal, Allamaneni, 2004)**.

There is an ongoing debate on the role of antioxidant supplementation in both male and female infertility. Reports of antioxidant therapy in female infertility are few and antioxidant therapies have not resulted in modification of outcomes of many of the diseases investigated. These fields are interesting areas to pursue, especially using preventative approach mainly caused by the high cost of such infertility treatments. Also, the role of OS in fertility and subfertility is an area deserving of continued research **(Sekhon et al., 2010)**.

Human research investigating OS and dietary antioxidants can be especially challenging. It is not possible to retrospectively collect data on very early pregnancy loss since many women may have been unaware that they were pregnant. Furthermore, in the event a woman was aware that she was pregnant, retrospective dietary data may be biased by the knowledge of a pregnancy loss **(Bunin et al., 2001)**. *In vitro* studies of humans and other mammals offer promising insight, but the nature of these experiments can introduce additional OS not found *in vivo*. They will prove the safety and effectiveness of antioxidants and could improve the maternal and fetal outcomes. Successful management of infertility in the ART scenario depends on overcoming OS in the *in vitro* conditions. The results of the studies reviewed need to be validated by larger randomized, double-blind, case-controlled trials **(Agarwal, Gupta, 2006)**.

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