International Journal of Farmacia

Journal Home page: www.ijfjournal.com

Nerveforte EC tablets: a super antioxidant maintaining optimum Male & Female fertilty

GovindShukla, Sangeetakumari, Dr,S.S.Dhingra, Dr.Indu Prasad, Jaspreet Singh, Mrs.AnuRawat, Mrs.Vijay laxmi, Sandeep, GopalSharma

Elaryk Pharmaceuticals, Makers of Nerveforte-Ec Tablets F212/2 F-Block Vishnu Garden New Delhi. India

Corresponding Author: Jaspreet Singh

ABSTRACT

Oxidants are highly unstable molecules that attack every chemical substance they come into contact. Oxidants modify the macromolecules both structurally and functionally. Body has defense mechanisms against oxidants in the form of both enzymatic and non-enzymatic antioxidants. Reactive oxygen species (ROS) are a group of oxidants formed during oxygen metabolism. ROS appears to be involved in the pathogenesis of many human diseases. In reproductive medicine, ROS have both physiological and pathological role in male and female reproduction. Oxidative stress develops when the generation of ROS overwhelms the scavenging capacity of antioxidants. Oxidative stress causes damage to spermatozoa, oocyte and embryos. It appears to play a role in both natural and in vitro fertilization and pregnancy. The patients with oxidative stress may benefit from the strategies to reduce oxidative stress by treatment with potent antioxidant therapy with Nerveforte-EC tablets. The present paper Reviews the Role of **NERVEFORTE-EC TABLETS** in maintaining optimum Male & Female fertilty.

Keywords: Oxidants, Antioxidants, **NERVEFORTE-EC TABLETS**, Infertility - Male & female, Spermatozoa, Reactive oxygen species, Free radicals.

INTRODUCTION

Infertility can be defined as a lack of pregnancy after one 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.

Recent research on the role of reactive oxygen species (ROS) in human infertility has received a great deal of interest from the scientists and medical practitioners [1-3].

Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are oxygen derived molecules, which are formed as intermediary products and are a class of powerful oxidants in the human body. ROS include superoxide anion (O2), hydrogen peroxide (H2O2) and hydroxyl radical (OH.). Some cells Posses specific mechanisms to produce ROS that are required for cellular functions in low concentrations [4]. Aerobic environment is a constant source of ROS through in vivo mechanisms such as electron leakage during biologic oxidations, and by physical activation of oxygen by external agents such as irradiation, e.g. UV sunlight. 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 [5].

Antioxidant Defense system against ROS

Organisms have developed efficient protective mechanisms against excessive accumulation of ROS. ROS are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase and glutathione peroxidase/reductase, and numerous non-enzymatic antioxidants such as vitamin C, vitamin E, vitamin A, pyruvate, glutathione, taurine and hypotaurine [7]. In a healthy body, pro-oxidants and antioxidants maintain a ratio and a shift in this ratio towards pro-oxidants gives rise to oxidative stress. This oxidative stress may be either mild or severe depending on the extent of shift. Whenever ROS levels become pathologically elevated, antioxidants begin to work and help minimize the oxidative damage, repair it or prevent it altogether. The male and female genital tracts are rich in both enzymatic and non-enzymatic antioxidants [7-10]. Vitamins C and E act as chain-breaking antioxidants and thus prevent the propagation of peroxidative process.

ROS AND CELL INJURY

Lipid peroxidation

ROS can attack polyunsaturated fatty acids in the cell membrane leading to a chain of chemical reactions called lipid peroxidation. Fatty acid breakdown results in the formation of various oxidatively modified products, which are toxic to cells and are finally converted into stable end products. The spermatozoa membrane contains large amounts of polyunsaturated fatty acids [11], which maintain its fluidity. Peroxidation of these fatty acids leads to the loss of membrane fluidity and a reduction 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 [5].

DNA damage

Susceptibility of DNA to oxidative damage is indicated by the presence of oxidatively modified substances like 8-hydroxy-2-deoxyguanosine.

Deoxyribonucleic acid bases and phosphodiester backbones are sites that are susceptible to peroxidative damage. High levels of ROS mediate the DNA fragmentation that is commonly observed in the spermatozoa of infertile men [12, 13]. 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 [14]. Various types of DNA abnormalities occur in sperm that have been exposed to ROS artificially. These abnormalities include base modification, production of base-free sites, deletions, frame shifts, DNA cross-links and chromosomal rearrangements [15, 16]. Patients with high levels of oxidative stress in their seminal fluid were found to have sperm with multiple single and double DNA strand breaks [17]. A biomarker for oxidative DNA damage, 8- hydroxy-2-deoxyguanosine, can be used to determine the extent of ROS-induced DNA damage.

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. 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 [3]. The process of apoptosis may also be accelerated by ROS-induced DNA damage and ultimately may lead to a decline in sperm count [6].

MEASUREMENT OF OXIDATIVE STRESS

Oxidative stress can be estimated directly or indirectly. The direct measurement of ROS is by using electron spin resonance method and is used sparingly in reproductive medicine. Indirect tests measure oxidatively modified products. Chemiluminescence is a common method used and is based on emission of light on chemiluminescent reaction between ROS and reagent (luminal/lucigenin). The amount of light emitted is quantified and measured by a luminometer.

Lipid peroxidation end products like malondialdehyde, lipid hydroperoxides, and

conjugated dienes are commonly used to assess the oxidative stress.

Other methods are measurement of protein and DNA oxidation products, and changes in status of antioxidants. Flow cytometry is also being used to measure the individual ROS radicals [18].

ROS-TAC score

It is a concept to represent the oxidative stress status of individual more accurately.

This score accommodate for the variations in both ROS and TAC (total antioxidant capacity) values (19). Fertile men tend to have high ROS-TAC scores whereas infertile men generally have significantly lower scores. ROS levels can also be measured directly in neat semen, thereby offering yet another measure of oxidative stress.

Role of Oxidative Stress in Male Infertility

The presence of free radicals in the spermatozoa was reported by MacLeod 50 years ago [20]. Because spermatozoa lack cytoplasmic enzymes, they often are unable to prevent oxidative damage by these free radicals. This is one of the features that make spermatozoa highly susceptible to peroxidative damage.

Most cytoplasmic enzymes are extruded during the final stages of the sperm maturation process, which enables sperm to attain their characteristic morphology [21].

Nature compensated for this deficiency by providing an array of antioxidants in the seminal plasma.

Sources of ROS

Morphologically abnormal spermatozoa and leukocytes are the major sources of ROS in the male reproductive tract. Even though mature spermatozoa may not produce pathologically significant levels of ROS, oxidative damage may occur in the epididymis and seminiferous tubules where they are in close contact with the immature, ROS producing spermatozoa and leukocytes [22].

ROS production is elevated in patients who have a large percentage of spermatozoa with excess residual cytoplasm in the midpiece [22]. Excess residual cytoplasm contains enzymes such as glucose-6-phosphate dehydrogenase and creatine phosphokinase, which are linked with generation of ROS and defective sperm function. ROS may be generated at the level of plasma membrane (NADPH-oxidase system) (23) or mitochondria (NADH-dependent oxido-reductase) [24].

Human spermatozoa generate O2 [25], which spontaneously or enzymatically dismutates to H2O2. In the presence of metal ions (iron)- O2 (and H2O2 together produces the more harmful oxidant, OH.. Neutrophils and macrophages are the major source of oxidants in the reproductive tract [26, 27]. During inflammation and infection, activated leukocytes can produce significantly higher amounts of ROS than non-activated leukocytes [28]. The ROS production in leukocytes is through NADPH oxidase enzyme. Even though ROS is released as part of defense mechanism in to the reproductive tract, it can damage surrounding spermatozoa, especially when antioxidant systems are overwhelmed. The importance of leukocyte contamination in producing ROS is well observed in Percoll-washed spermatozoa where a small number of leukocytes produce ROS. Increased levels of seminal leukocytes may also stimulate human spermatozoa to produce ROS. Such stimulation may be mediated via direct cell-cell contact or by soluble products released by leukocytes [27].

Mechanism of loss of sperm function by ROS

ROS may affect the quality and number of spermatozoa reaching the ovum in the female reproductive tract. In addition, ROS impair the fertilization process by preventing the initiation of sperm-oocyte fusion events [14]. Finally, ROS can impair embryo development and affect the health of offspring by damaging sperm DNA [16].

Impairment of standard semen parameters

Motility is a very important attribute unique to spermatozoa in entire human cells. Motility is indispensable to the spermatozoa, as it has to travel the female reproductive tract to reach the site of fertilization. Studies found that levels of ROS correlate with motility of spermatozoa [30, 31]. In vitro studies showed that the impaired motility may be a temporary event or permanent phenomena.

Excessive ROS causes ATP to deplete rapidly resulting in decreased phosphorylation of axonemal proteins and cause transient impairment of motility [32]. Peroxidative damage to the sperm membrane and axonemal proteins appears to be the cause of permanent impairment in sperm motility. ROS appears to play a role in the apoptosis of spermatozoa by activating caspases. Under normal conditions, abnormal sperm undergo apoptosis, which minimizes

their presence in the semen. The severity of oligozoospermia has been correlated with excessive levels of ROS [33]. ROS may stimulate the process of apoptosis, resulting in the death of spermatozoa and decreased sperm count [6]. Patients with a low sperm count have a reduced chance of initiating a pregnancy.

Impairment of sperm-oocyte fusion

A minimal amount of ROS is required for the normal sperm-oocyte fusion. Spermatozoa and oocyte has inbuilt mechanism to prevent excessive production of ROS at the time of sperm-oocyte fusion, this may be by the release of SOD (superoxide dismutase) [35]. 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 [36, 37].

Sperm DNA damage

Sperm DNA contributes the half of genomic material to the offspring. Thus, normal sperm genetic material is required for fertilization, embryo and fetus development and postnatal child well being [16, 38]. A recent study showed decreasing likelihood of pregnancy with increasing levels of 8-hydroxy-2-deoxyguanosine, an indicator of oxidative damage to DNA [39]. The percentage of sperm with DNA damage is negatively correlated with the fertilization rate [12]. Oocytes can repair DNA damage to some extent, but when the damage is severe, embryo death and miscarriages can occur. The affect of ROS on DNA integrity has become the focus of recent attention due to widespread use of assisted reproduction techniques (ART) such as intracytoplasmic injection (ICSI). In natural pregnancy, oxidative damage to the sperm membrane may ensure that spermatozoa with damaged DNA lose their ability to fertilize an oocyte. However, sperm with DNA damage can potentially be injected into an oocyte in the ICSI resulting in fertilization and pregnancy which may progress to live birth with congenital abnormalities [34].

ROS & Male infertility

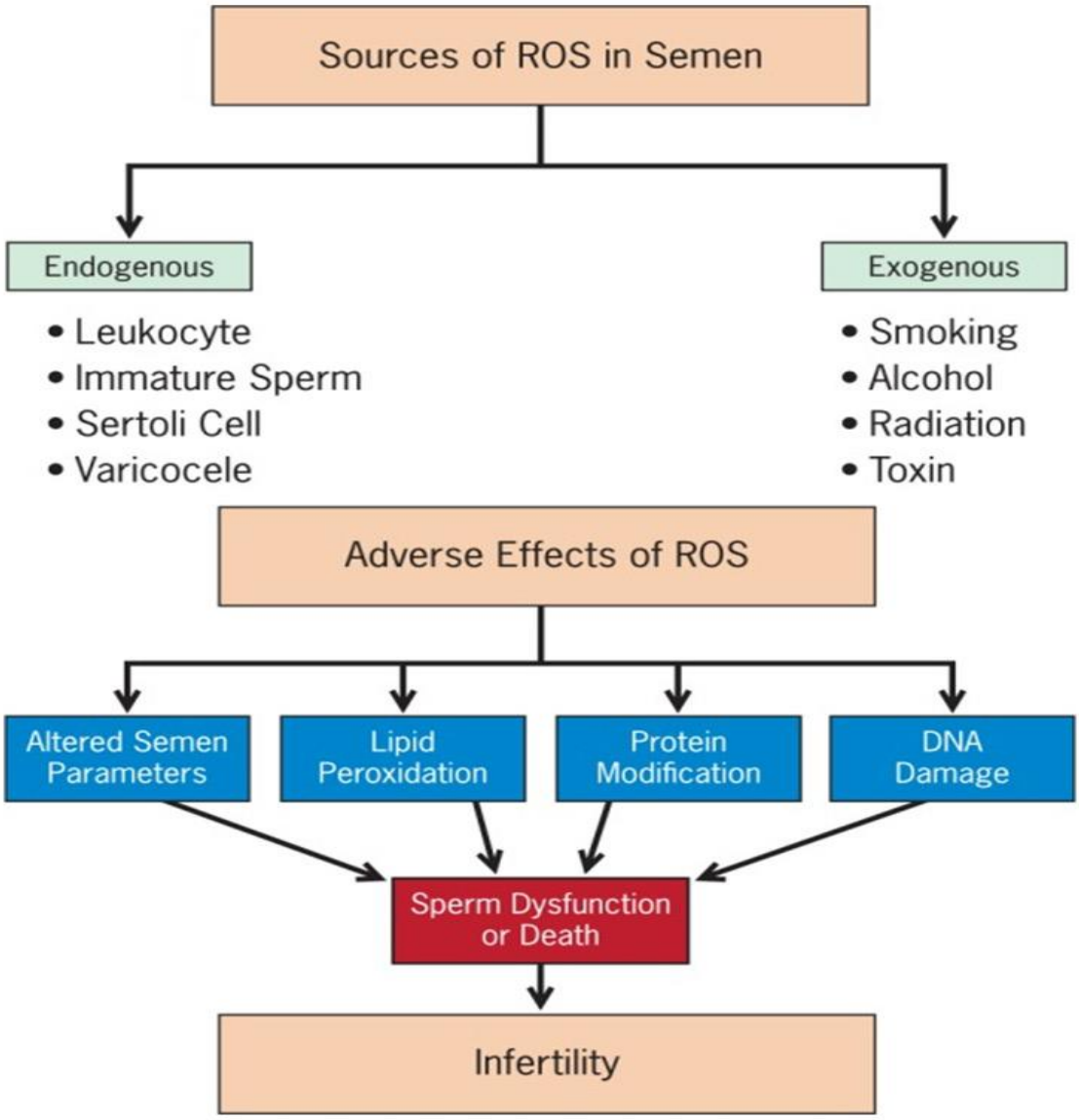

Figure 1: Common sources of excessive reactive oxygen species (ROS) in semen and their deleterious effect

Many clinical conditions were found to be associated with increased oxidative stress [33]. Infections and inflammations involving the male reproductive tract are obvious conditions associated with oxidative stress in view of excessive generation of ROS by leukocytes [40-42]. Very high percentage of spinal cord injury patients were reported to have elevated levels of oxidative stress [43, 44].

Mechanism of infertility in patients with varicocele is poorly understood and ROS is postulated as a possible mediator [45, 46]. Elevated levels of ROS and depressed levels of TAC were associated with varicocele [47-49]. Patients who underwent vasectomy reversal also had high levels of reactive oxygen species [50, 51]. A history of smoking was associated with high levels of oxidative stress [52].

ROLE OF ROS IN FEMALE INFERTILITY

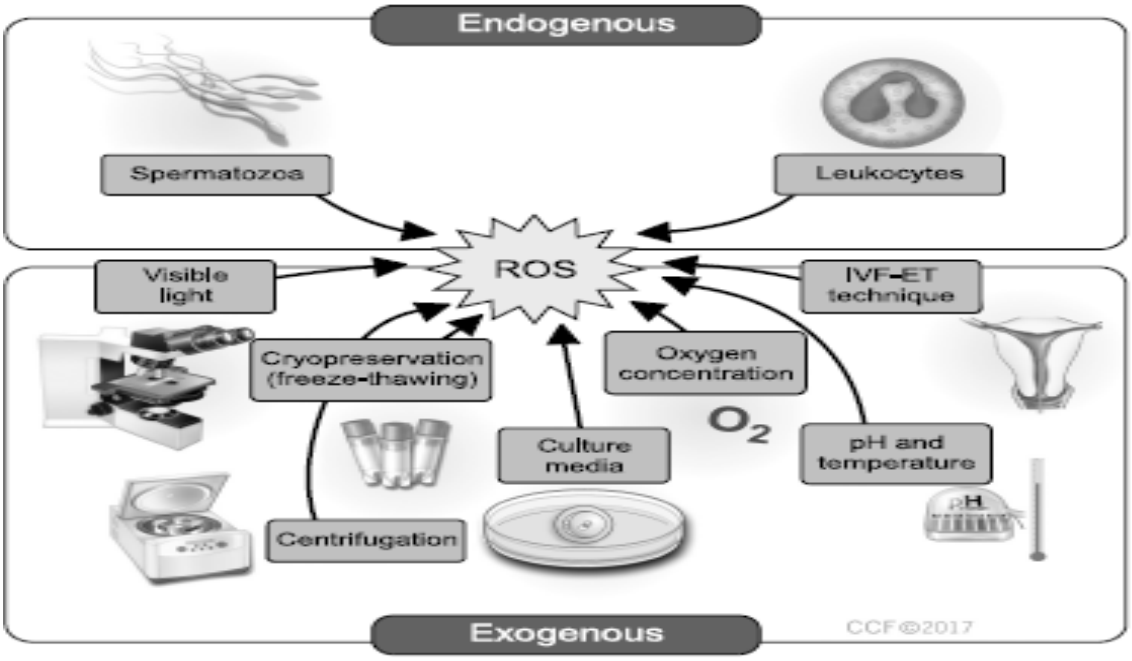

Fig. 2. Sources of reactive oxygen species (ROS) in the assisted reproduction setting. IVF-ET: *in vitro* fertilization-embryo transfer

Many studies reported the presence of oxidative and antioxidant systems in various female reproductive tissues [53-57]. ROS appears to have physiological role in female reproductive tract in many different processes such as: oocyte maturation, fertilization, luteal regression, and endometrial shedding [58, 59]. ROS levels in follicular fluid may be used as markers for predicting the success of in vitro fertilization (IVF) [3].

Whenever there is imbalance in the levels of ROS and antioxidants- damage can occur to oocytes and embryos through various pathological mechanisms.Oxidative stress can affect the female fertility potential in number of ways. It may affect the ovulation, fertilization, embryo development and implantation. The sources of ROS in Graffian follicle may be macrophages, neutrophils and granulosa cells. 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 [60]. Another study reported that baseline TAC levels were higher in follicles whose oocytes fertilized successfully [61]. 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 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 [57, 62]. High levels of malondialdehyde and low levels of antioxidants in the peritoneal fluid were reported in patients with unexplained infertility compared to controls [63].

Oxidative stress & its role in endometriosis

Oxidative stress is postulated as one of the possible mechanism of endometriosis. [65]. The endometrial tissue has multiple cells like macrophages, red blood cells, which can generate ROS. Studies of women with endometriosis have suggested that peritoneal macrophages are responsible for increased production of ROS or increased expression of xanthine oxidase in endometrial cells [65, 66]. High levels of oxidatively modified substances in peritoneal fluid and ectopic endometrial

tissue were reported [67]. Altered expression of defensive antioxidant enzymes and low levels of vitamin E were reported in patients with endometriosis [68].

Effect of ROS 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 [71, 72]. ROS not only alters most types of cellular molecules but also induces early embryonic developmental block and retardation [56]. High levels of ROS and apoptosis were reported in fragmented embryos compared to non-fragmented embryos [73].

Effects of Oxidative stress on in-vitro fertilization

DNA damage induced by oxidative stress has important clinical implications in the context of assisted reproduction. Spermatozoa selected for ART most likely originate from an environment experiencing OS, and a large percentage of these sperm may have damaged DNA (2). There is a strong possibility that spermatozoa with damaged DNA may be used during ART [16], 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 [77, 78]. Estimating ROS levels may help predict the success rate of assisted reproduction procedures.

ROS and Sperm Preparation

A possible source of ROS in ART media is during the preparation of semen. Sperm preparation is necessary to enhance and maintain sperm quality and function after ejaculation before the semen specimen can be used for ART procedures [79]. The production of ROS may be due to either 1) activation of spermatozoa by centrifugation process, 2) absence of antioxidant rich seminal plasma, or 3) because of minimal contamination of ROS produced by leukocytes and abnormal spermatozoa. The small amount of ROS produced may not decrease motility but can still cause DNA damage (29). A proper sperm preparation method should be selected so as to decrease the production of ROS.

THERAPEUTIC TREATMENT STRATEGIES AGAINST ROS

In both male and female reproduction, oxidative stress appears to be due to increased generation of ROS rather than a depletion of antioxidants. It is important to identify the source of increased ROS generation [80]. The underlying etiological factor for abnormal leukocyte infiltration (e.g. leukocytospermia, inflammation, infection, smoking) should be determined. Patients with history of smoking should be advised to stop smoking. Any exposure to drugs, toxic substances and radiation should be checked and patients advised to stop exposure to them. Infections of the reproductive tract should be treated with appropriate antibiotics.

Initially, specific therapeutic options directed against the etiological cause of raised ROS should be tried. Patients with reproductive tract infection should be treated with antibiotics. Antiinflammatory agents may help patients with persistent leukocytospermia and elevated levels of cytokines.

After treating the primary cause (such as varicocele), patients can be advised to take antioxidant supplementation. Antioxidants can be started directly when a specific etiology cannot be identified (idiopathic infertility).

Male infertility

Semen analysis should be repeated after a full spermatogenic cycle in those men showing large number of abnormal spermatozoa with excessive cytoplasm in the mid piece during a routine analysis. This can help distinguish between a temporary disturbance in spermatogenesis and a permanent defect in spermatogenesis.

Varicocelectomy may remove an unknown stimulus of ROS generation. Even though there is no definitive consensus on the use of antioxidants, many in vitro and in vivo studies have shown that they improve semen quality and fertility [80]. Some studies showed improvement in terms of pregnancy rate after antioxidants supplementation.

Oral vitamin E is an antioxidant favored by many researchers and clinicians. Oral administration of 300 mg twice a day of vitamin E in a randomized double blind placebo controlled trial showed significant improvement of pregnancy rates (21%; 11/52) in infertile (asthenozoospermic) patients, while resulting in lack of pregnancies in the placebo group. This study also found significant improvement in sperm

motility, and reduced lipid peroxidation levels after vitamin E supplementation [81].

A combination of vitamin E and selenium in oligoasthenoteratozoospermic (OAT) patients resulted in significant improvement in sperm motility, viability and morphology [82].

Treatment may be more appropriate if antioxidants are given to the patients with raised ROS levels.

The combination therapy of vitamins A plus E and essential fatty acids significantly reduced ROS and improved pregnancy rates [84].

Oral administration of 200 mg of vitamin C, 200 mg of vitamin E and 400 mg of GSH for 2 months significantly improved serum levels of antioxidants and relatively decreased sperm DNA damage [13].

Female infertility

There are few studies on the role of antioxidants in female infertility (89, 90). Both the studies reported higher pregnancy rate with vitamin C supplementation compared to the control group. In vivo antioxidants may be helpful in infertile women who smoke, as history of smoking is associated with high levels of oxidative stress [55].

Use of antioxidants in IVF media appears to be useful in improving the pregnancy rates. Higher implantation and pregnancy rates were found when antioxidant supplemented media was used rather than standard media without antioxidants [91].

Antioxidants, especially vitamin C, can improve the blastocyst development rate in a mouse embryo model [92].

In ART procedures, sperm preparation techniques separate mature spermatozoa and thus minimize the interaction between ROS producing cells in semen (e.g. leukocytes, immature abnormal spermatozoa) and normal spermatozoa. Density gradient separation and swim-up methods are commonly used sperm preparation methods. Adding antioxidants to the sperm preparation media may help prevent ROSinduced damage and preserves the quality of spermatozoa during ART procedures.

COMPOSITION OF NERVEFORTE-EC TABLETS

- Methylcobalamine ------1500 mcg
- Omega-3 FattyAcid----- 150 mg
- Vitamin A----------5000 IU
- Vitamin C----------150 mg
- Vitamin E----------200 IU
- ElementalZinc---------- 20 mg
- Selenium----------70mcg
- Folic Acid----------5 mg

MECHANISM OF ACTION OF EACH INGREDIENTS OF NERVEFORTE-EC TABLETS

Methylcobalamine

Methylcobalamine is a water-soluble vitamin that helps in the proper functioning of the brain and nervous system. It's the largest vitamin B and is vital for metabolizing the body cells. Besides, it plays a major role in producing the genetic components DNA and RNA of the egg as well as the sperm.

Deficiency of Methylcobalamine can affect the fertility of both men and women. When body lacks B12, it can result in serious consequences, such as weakness, memory loss, infertility, anaemia, neurological or psychiatric problems.

Methylcobalamine plays a significant role in generation and maturation of the sperms. Men suffering from fertility issues should consider consuming foods rich in Vitamin B12 to meet their body's requirement of Vitamin B12. A deficiency of this vitamin may negatively affect the sperm count or lead to following issues:

- Low sperm count
- DNA damage in sperm cells
- Low motility of sperm
- Loss of libido
- Premature ejaculation

For a woman with a deficiency in Methylcobalamine trying to conceive is difficult. Even if a woman with Methylcobalamine gets pregnant, the chances of foetal loss are higher. The lack of B12 possibly interferes with the reproduction and contributes to below problems:

- Abnormal ovulation
- Disruption in normal cell division
- Impaired development of the egg
- Difficulty implanting the fertilized ovum in the uttering lining

Omega-3 FattyAcid

Omega-3 acids have been shown to help fertility by helping to regulate hormones in the body, increase cervical mucus, promote ovulation, and overall

improve the quality of the uterus by increasing the blood flow to the reproductive organs.

Omega-3 fats also contain two acids that are crucial to good health: DHA and EPA. These two acids have been shown to help many forms of disease. Low levels of DHA have been linked to depression and other mental health issues. During pregnancy, a lack of DHA may be associated with premature birth, low birth weight, and hyperactivity in children.

Omega 3 EFAs help the body to

- Regulate hormones
- Increase the blood flow to the uterus
- Reduce sensitivity to the hormone prolactin, which can suppress ovulation.
- **Increases egg white cervical mucus**, which is needed to help the sperm reach the egg.
- Helps menstrual cycle to become normalized.

Essential Fatty Acids and Pregnancy

It is important to have a plentiful supply of essential fatty acids before becoming pregnant and to continue to eat fish or take fish oil supplements during pregnancy to maintain optimal health.

During pregnancy, essential fatty acids are important to baby's brain, eye, and heart development.

- EPA is necessary to grow a healthy circulatory system.
- DHA is an important component for a healthy central nervous system.

These EFAs support retinal development and visual acuity. Studies are showing that babies born to mother with high levels of DHA do better with visual acuity and visual learning tests. With optimal development that these nutrients provide, studies show that baby will enjoy enhanced attention span and cognitive function after the birth as well, which will help them learn more effectively.

Having a sufficient level of these fats in our system is thought to help prevent miscarriage and premature delivery. Premature birth occurs in as many as 6 – 10% of births and increases the risk of physical and learning disabilities.

Omega-3s have been shown to be instrumental in preventing complications in pregnancy like pre-eclampsia, which can endanger the lives of both mother and baby. It may also lower the risk of postpartum depression after your baby is born.

It is important to have a plentiful supply of essential fatty acids before becoming pregnant and to continue to eat fish or take fish oil supplements during pregnancy to maintain optimal health.

Essential Fatty Acids and Men's Fertility

For men, essential fatty acids can also make a big difference,

- Fatty acids **improve the circulation to the genitals**, which help support the prostate and the other elements needed for reproduction.
- It helps lower blood pressure, which helps with erectile dysfunction.
- Omega 3s are **important to the production of sperm**

Essential fatty acids and cholesterol are primary ingredients in sperm cell membranes.

DHA, one of the fatty acids contained in fish oil, has a significant impact on the viability and health of sperm. When there are not enough fatty acids present, cholesterol replaces the needed fatty acid in the sperm membrane. This prevents sperm from proper maturation. This, in turn, helps create more free radicals, which damage any healthy sperm that may be present.

By increasing the intake of essential fatty acids through the consumption of fish or fish oil supplements, the resulting sperm are healthier, have better motility, and the chances of a successful conception increase.

Vitamin A

Carotenoids are a group of fat-soluble organic compounds found mainly in yellow, red, orange and pink vegetables. These carotenoids are precursor of vitamin A. Retinal is formed from them in the gastrointestinal tract, before being converted to retinol, the most important component of vitamin A.

Carotenoids are natural antioxidants that protect cell membrane integrity, regulate epithelial cell proliferation, and are involved in the regulation of spermatogenesis. Moreover, in rats retinoids have various effects on fetal and neonatal Sertoli, germ and Leydig cell. Carotenoid deficiency in the diet can lead to a reduction in sperm motility. Studies conducted on bulls have shown that retinol might stabilize sperm acrosomal membrane when oxidative stress increases because of the high temperature. In humans, lower serum retinol concentration has been correlated to a worse sperm quality. Hence, vitamin A administration could be a therapeutic choice for the treatment of human male infertility.

Vitamin C (Ascorbic Acid)

Vitamin C has a 10-fold higher concentration in seminal plasma than in the serum. It has a more powerful antioxidant action when peroxyradicals are present in the aqueous phase than in the lipid membrane. In mice, at a concentration equivalent to the human therapeutic dose (10mg/Kg), it is able to reduce MDA concentration, increasing sperm count and the proportion of normal sperm population.

Vitamin E

A powerhouse of antioxidants, it can help fight toxins.

Benefits of Vitamin E for Men

1. **Improves motility of the sperm:** Good motility of sperms is a necessity, as sperms have to cross over a certain distance, in order to fertilize an egg. This nutrient aids in the same. It does this by counteracting the damage of reactive oxygen species (ROS) on sperm motility.
2. **An increase in the sperm count:** One of the most common causes of infertility in men is a low sperm count. Ideally, the count is considered less if one millilitre of your semen consists of less than 20 million sperms. In most cases, a decrease in the sperm count is due to infections and damage – something that this nutrient can protect you against. It can prevent infections, damage and act as a protective barrier.
3. **Boosting your sex drive:** Research suggests that to increase the levels of testosterone in your body, you must consume foods that are rich in vitamin E. An increase in this hormone can boost sexual interest and sexual stamina. Since a depressed sex drive can also cause infertility, incorporating this nutrient into your diet can improve your sex drive.
4. **Better IVF results:** Research suggests that men who had a regular intake of this showed an increase of up to 29% in the rate of fertilization, as opposed to men who had an insufficient consumption of this nutrient. It does this by improving the quality of sperms.

Benefits of Vitamin E for Women

Here are a few reasons why vitamin E for women's fertility

1. **Thickening of the uterine lining:** A thin uterine lining can be a major causal factor of infertility in women. Consuming close to 600mg a day can .increase the blood flow in the radial artery, thereby increasing the thickness of the uterine lining.
2. **Treats fibrocystic breast disease:** Fibrocystic breast disease is a condition where breasts develop benign tumours, that during menstruation, become swollen and painful. This nutrient can help in the reduction of these tumours.
3. **Treat polycystic ovary syndrome (PCOS):** Polycystic ovary syndrome is a common condition that affects close to one in every ten women. This syndrome causes a hormonal imbalance and slows down the rate of metabolism. It can help in reversing the effects of PCOS.
4. **Protects the amniotic sac:** The amniotic sac, which contains the amniotic fluid, is what protects and contains the foetus in the womb. In some cases, the amniotic sac suffers from the rupture of the amniotic sac. With this mineral, the membranes are made stronger, thereby preventing the likelihood of an amniotic sac rupture.

Zinc

Zinc is a component of over 200 enzymes involved in the biosynthesis of nuclear acids, proteins and the process of cell division. It has been reported to normalize oxido-sensitive indices and catalase-like activity in the seminal fluid of astheno-zoospermic patients.

Zinc is given orally at the dose of (220mg) once or twice a day for 3 to 4 months, alone or in addition to folic acid (5mg daily). In combined therapies, it is administered at the dose of at least 10mg a day.

Selenium

Selenium is a micronutrient essential for normal testicular development, spermatogenesis, sperm motility and function. The lack of selenium has been correlated to seminiferous epithelium atrophy, disorders of spermatogenesis, maturation of spermatozoa in the epididymis, testis volume reduction, decreased sperm motility and altered sperm morphology (mainly in the head and in the midpiece) . The exact mechanism by which selenium reduces oxidative stress and improves sperm parameters is still controversial. Its action seems to be mediated by seleno-enzymes, such as GPXs. Selenium is given orally at a dosage that ranges from (80 to 300µg) once

a day, alone or in combination with antioxidants, for at least 3 months.

Folic acid

Folic acid is a synthetic form of vitamin B9, one of a group of eight water-soluble vitamins that are fundamental to cell metabolism. In its naturally occurring form, vitamin B9 is known as folate.

Folic Acid and Female Fertility

Folic acid can have a powerful effect on female fertility. In fact, specially formulated folic acid supplements are generally recommended to women who are planning to become pregnant, whether they struggle with infertility issues or not. These supplements, along with foods rich in folate and folic acid, should be regularly taken immediately upon the decision to pursue pregnancy, and preferably several months before conception occurs.

A folic acid regimen is particularly important to women with fertility problems, not only because it promotes fertility, but also because stress causes the body to need more folate. Once pregnancy is achieved, folic acid remains essential, particularly during the first trimester, as it reduces the risk of miscarriage and congenital defects.

Although it is rare that someone would suffer ill effects from taking too much folic acid, it remains a possibility. Therefore, it is extremely important to use folic acid as part of your fertility plan exactly as recommended by our doctors.

Folic Acid and Male Fertility

In recent years, there have been multiple studies demonstrating a link between folic acid intake and male fertility. Folic acid can actually improve the quality of a man's sperm while lowering the risk of sperm abnormalities that could interfere with conception. In particular, a study led by Dr. Wai Yee Wong of the University Medical Center Nijmegen in the Netherlands found that men struggling with male factor infertility who had been taking folic acid and zinc supplements for 26 weeks experienced a 74 percent increase in the amount of normal sperm in their semen.

NERVEFORTE-EC TABLETS IN MALE INFERTILITY

NERVEFORTE-EC Tablets Protects sperm from oxidative damage. Significantly improves sperm quality. Increases sperm count. Concentration, Motility,Morphology

NERVEFORTE-EC TABLETS IN FEMALE INFERTILITY

NERVEFORTE-EC TabletsProtects ovum & gametes,Ensures successful nidation,Improves pregnancy rate & outcome. Reduces chances of complications.NERVEFORTE-EC Tablets Prevents & reduces chances of pre-eclampsa & PIH,Helps in adequate growth of foetus - reduces IUGR chances ,Reduces risk of abortions. Helps in successful pregnancy.

Other Clinical Indications

Studies have also investigated the relationship and/or use of **NERVEFORTE-EC Tablets** for cataracts,longevity, malaria, digestive-tract cancers,immune modulation, Alzheimer's disease, and preeclampsia.

DOSAGE

- 1-2 Tablets daily with meals

INDICATIONS

NERVEFORTE-EC Tablets prevents **male infertility**

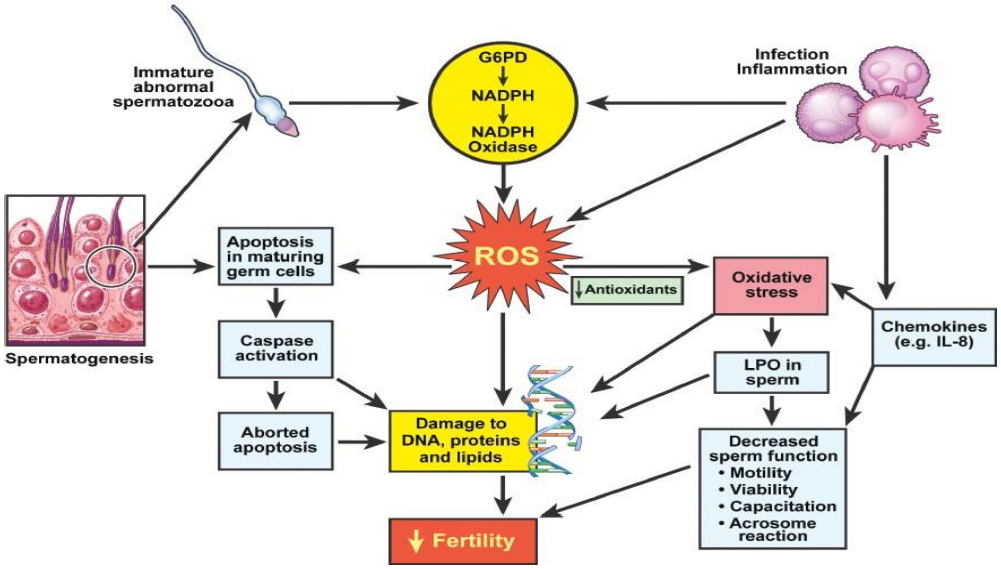

Fig 8: Pathophysiology of Male Infertility

Oxidative stressis an important aspect in male infertility

- Infertile men have very high levels of semen ROS
- Spermatogenesis is very sensitive to oxidative stress
- Protects sperm from oxidative damage
- Significantly improves sperm quality. Increases sperm count. Concentration, Motility ,Morphology

NERVEFORTE-EC Tablets prevent Female infertility

- Protects ovum & gametes
- Ensures successful nidation
- Improves pregnancy rate & outcome
- Reduces chances of complications
- Prevents & reduces chances of pre-eclampsa & PIH
- Helps in adequate growth of foetus - reduces IUGR chances
- Reduces risk of abortions.

Supplement Facts

Presentation: Tablet

INDICATIONS

A Super Antioxidant maintaining optimum Male & Female fertilty

- **Contra-indications:** Product is contra-indicated in persons with Known hypersensitivity to any component of the product hypersensitivity to any component of the product.
- **Recommended usage :** *Adults:*1-2 tablet twice a day with water or liquid of choice twice daily
  "Do not exceed the recommended daily dose"
- **Administration:** Taken by oral route at anytime with food.
- **Precautions:** Do not exceed the recommended daily dose.
- **Warnings:** If you are taking any prescribed medication or has any medical conditions always consults doctor or healthcare practitioner before taking this supplement.
- **Side Effects:** Very Mild side effects like nausea, headache and vomiting in some individuals may be observed.
- **Storage:** Store in a cool, dry and dark place.

SUMMMARY & CONCLUSION

It is widely accepted that oxidative stress is a significant factor in the progression of diseases. Oxidative stress is associated with increased reactive oxygen species and is known to accelerate disease

formation since superoxide is converted to a toxic substance, namely hydrogen peroxide. This reaction is prevented by antioxidant enzymes, namely catalase, superoxide dismutase and glutathione peroxidase. Antioxidants are key prophylactic agents in preventing oxidation related cell damage . A large number of epidemiological and interventional studies have been investigated for the role of dietary antioxidant supplement in the prevention of life style disorders. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation of **NERVEFORTE-EC tablets** is now being recognized as an important means of improving free radical protection.

Presence of multiple antioxidant systems in the human body favors the use of antioxidants combination rather than single antioxidant supplementation. Supplementation of Super Antioxidant like Nerveforte-EC tablets may be beneficial in patients with infertility.

REFERENCES

[1]. Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. Urology 48, 1996, 835-50.

[2]. Sikka SC. Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. J Androl 25, 2004, 5-18.

[3]. Agarwal A, Saleh RA. Role of oxidants in male infertility: rationale, significance, and treatment. Urol Clin North Am 29, 2002, 817-27.

[4]. Saleh RA, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice. J Androl 23, 2002, 737-52.

[5]. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 79, 2003, 829-43.

[6]. Halliwell B. Tell me about free radicals, doctor: a review. J R Soc Med 82, 1989, 747-52.

[7]. Gavella M, Lipovac V, Vucic M, Rocic B. Superoxide anion scavenging capacity of human seminal plasma. Int J Androl 19, 1996, 82-90.

[8]. Jozwik M, Kuczynski W, Szamatowicz M. Nonenzymatic antioxidant activity of human seminal plasma. Fertil Steril 68, 1997, 154-7.

[9]. Smith R, Vantman D, Ponce J, Escobar J, Lissi E. Total antioxidant capacity of human seminal plasma. Hum Reprod 11, 1996, 1655-60.

[10]. Zini A, Garrels K, Phang D. Antioxidant activity in the semen of fertile and infertile men. Urology 55, 2000, 922-6.

[11]. Jones R, Mann T, Sherins R. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. Fertil Steril 31, 1979, 531-7.

[12]. Sun JG, Jurisicova A, Casper RF. Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol Reprod 56, 1997, 602-7.

[13]. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertil Steril 68, 1997, 519-24.

[14]. Aitken RJ, Baker MA, Sawyer D. Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. Reprod Biomed Online 7, 2003, 65-70.

[15]. Duru N, Morshedi M, Oehninger S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. Fertil Steril 74, 2000, 1200 -7.

[16]. Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod Update 9, 2003, 331-45.

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

[18]. Marchetti C, Obert G, Deffosez A, Formstecher P, Marchetti P. Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. Hum Reprod 17, 2002, 1257-65.

[19]. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ, Jr., Agarwal A. The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Hum Reprod 14, 1999, 2801-7.

[20]. MacLeod. The role of oxygen in the metabolism and motility of human spermatozoa. Am J Physiol 138, 1943, 512.

[21]. Aitken J, Fisher H. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. Bioessays 16, 1994, 259-67.

[22]. Gil-Guzman E, Ollero M, Lopez MC, Sharma RK, Alvarez JG, Thomas AJ, Jr., et al. Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. Hum Reprod 16, 2001, 1922-30.

[23]. Aitken RJ, Buckingham D, West K, Wu FC, Zikopoulos K, Richardson DW. Differential contribution of leucocytes and spermatozoa to the generation of reactive oxygen species in the ejaculates of oligozoospermic patients and fertile donors. J Reprod Fertil 94, 1992, 451-62.

[24]. Gavella M, Lipovac V. NADH-dependent oxidoreductase (diaphorase) activity and isozyme pattern of sperm in infertile men. Arch Androl 28, 1992, 135-41.

[25]. Alvarez JG, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl 8, 1987, 338-48.

[26]. Shekarriz M, Sharma RK, Thomas AJ, Jr., Agarwal A. Positive myeloperoxidase staining (Endtz test) as an indicator of excessive reactive oxygen species formation in semen. J Assist Reprod Genet 12, 1995, 70-4.

[27]. Ochsendorf FR. Infections in the male genital tract and reactive oxygen species. Hum Reprod Update 5, 1999, 399-420.

[28]. Plante M, de Lamirande E, Gagnon C. Reactive oxygen species released by activated neutrophils, but not by deficient spermatozoa, are sufficient to affect normal sperm motility. Fertil Steril 62, 1994, 387-93.

[29]. Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z, et al. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod 59, 1998, 1037-46.

[30]. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril 57, 1992, 409-16.

[31]. Armstrong JS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, Sikka SC. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. Free Radic Biol Med 26, 1999, 869-80.

[32]. De Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. J Androl 13, 1992, 379-86.

[33]. Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ, Agarwal A. Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. Fertil Steril 73, 2000, 459-64.

[34]. Aitken RJ. The Amoroso Lecture. The human spermatozoon--a cell in crisis? J Reprod Fertil 115, 1999, 1-7.

[35]. Maiorino M, Ursini F. Oxidative stress, spermatogenesis and fertility. Biol Chem 383, 2002, 591-7.

[36]. Aitken RJ, Irvine DS, Wu FC. Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. Am J Obstet Gynecol 164, 1991, 542-51.

[37]. Griveau JF, Le Lannou D. Reactive oxygen species and human spermatozoa: physiology and pathology. Int J Androl 20, 1997, 61-9.

[38]. Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi PG, Bianchi U. Origin of DNA damage in ejaculated human spermatozoa. Rev Reprod 4, 1999, 31-7.

[39]. Loft S, Kold-Jensen T, Hjollund NH, Giwercman A, Gyllemborg J, Ernst E, et al. Oxidative DNA damage in human sperm influences time to pregnancy. Hum Reprod 18, 2003¸1265-72.

[40]. Pasqualotto FF, Sharma RK, Potts JM, Nelson DR, Thomas AJ, Agarwal A. Seminal oxidative stress in patients with chronic prostatitis. Urology 55, 2000, 881 5.

[41]. Potts JM, Sharma R, Pasqualotto F, Nelson D, Hall G, Agarwal A. Association of ureaplasma urealyticum with abnormal reactive oxygen species levels and absence of leukocytospermia. J Urol 163, 2000, 1775-8.

[42]. Vicari E. Effectiveness and limits of antimicrobial treatment on seminal leukocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. Hum Reprod 15, 2000, 2536-44.

[43]. de Lamirande E, Leduc BE, Iwasaki A, Hassouna M, Gagnon C. Increased reactive oxygen species formation in semen of patients with spinal cord injury. Fertil Steril 63, 1995, 637-42.

[44]. Padron OF, Brackett NL, Sharma RK, Lynne CM, Thomas AJ, Jr., Agarwal A. Seminal reactive oxygen species and sperm motility and morphology in men with spinal cord injury. Fertil Steril 67, 1997, 1115-20.

[45]. Naughton CK, Nangia AK, Agarwal A. Pathophysiology of varicoceles in male infertility. Hum Reprod Update 7, 2001, 473-81.

[46]. Allamaneni SSR, Naughton CK, Sharma RK, Thomas AJ, Jr., Agarwal A. Increased seminal reactive oxygen species levels in varicocele patients correlate with varicocele grade, not testis size. Fertil Steril 2004.

[47]. Hendin BN, Kolettis PN, Sharma RK, Thomas AJ, Jr., Agarwal A. Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. J Urol 161, 1999, 1831-4.
[48]. Barbieri ER, Hidalgo ME, Venegas A, Smith R, Lissi EA. Varicocele-associated decrease in antioxidant defenses. J Androl 20, 1999, 713-7.
[49]. Mostafa T, Anis TH, El-Nashar A, Imam H, Othman IA. Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. Int J Androl 24, 2001, 261-5.
[50]. Kolettis PN, Sharma RK, Pasqualotto FF, Nelson D, Thomas AJ, Jr., Agarwal A. Effect of seminal oxidative stress on fertility after vasectomy reversal. Fertil Steril 71, 1999, 249-55.
[51]. Shapiro RH, Muller CH, Chen G, Berger RE. Vasectomy reversal associated with increased reactive oxygen species production by seminal fluid leukocytes and sperm. J Urol 160, 1998, 1341-6.
[52]. Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas AJ, Jr. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. Fertil Steril 78, 2002, 491-9.
[53]. Jozwik M, Wolczynski S, Szamatowicz M. Oxidative stress markers in preovulatory follicular fluid in humans. Mol Hum Reprod 5, 1999, 409-13.
[54]. Attaran M, Pasqualotto E, Falcone T, Goldberg JM, Miller KF, Agarwal A, et al. The effect of follicular fluid reactive oxygen species on the outcome of in vitro fertilization. Int J Fertil Womens Med 45, 2000, 314-20.
[55]. Paszkowski T, Clarke RN, Hornstein MD. Smoking induces oxidative stress inside the Graafian follicle. Hum Reprod 17, 2002, 921-5.
[56]. Guerin P, El Mouatassim S, Menezo Y. Oxidative stress and protection against reactive oxygen species in the preimplantation embryo and its surroundings. Hum Reprod Update 7, 2001, 175-89.
[57]. Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR, et al. Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. Hum Reprod 17, 2002, 426-31.
[58]. Riley JC, Behrman HR. Oxygen radicals and reactive oxygen species in reproduction. Proc Soc Exp Biol Med 198, 1991, 781-91.
[59]. Sugino N, Karube-Harada A, Taketani T, Sakata A, Nakamura Y. Withdrawal of Ovarian Steroids Stimulates Prostaglandin F2alpha Production Through Nuclear FactorkappaB Activation via Oxygen Radicals in Human Endometrial Stromal Cells: Potential Relevance to Menstruation. J Reprod Dev 50, 2004, 215-25.
[60]. Paszkowski T, Traub AI, Robinson SY, McMaster D. Selenium dependent glutathione peroxidase activity in human follicular fluid. Clin Chim Acta 236, 1995, 173-80.
[61]. Oyawoye O, Abdel Gadir A, Garner A, Constantinovici N,Perrett C, Hardiman P. Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: relationship to outcome. Hum Reprod 18, 2003, 2270-4.
[62]. Wang Y, Sharma RK, Falcone T, Goldberg J, Agarwal A. Importance of reactive oxygen species in the peritoneal fluid of women with endometriosis or idiopathic infertility. Fertil Steril 68, 1997, 826-30.
[63]. Polak G, Koziol-Montewka M, Gogacz M, Blaszkowska I, Kotarski J. Total antioxidant status of peritoneal fluid in infertile women. Eur J Obstet Gynecol Reprod Biol 94, 2001, 261-3.
[64]. Bedaiwy MA, Falcone T. Peritoneal fluid environment in endometriosis. Clinicopathological implications. Minerva Ginecol 55, 2003, 333-45.
[65]. Ota H, Igarashi S, Hatazawa J, Tanaka T. Endothelial nitric oxide synthase in the endometrium during the menstrual cycle in patients with endometriosis and adenomyosis. Fertil Steril 69, 1998, 303-8.
[66]. Zeller JM, Henig I, Radwanska E, Dmowski WP. Enhancement of human monocyte and peritoneal macrophage chemiluminescence activities in women with endometriosis. Am J Reprod Immunol Microbiol 13, 1987, 78-82.
[67]. Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. Fertil Steril 77, 2002, 861-70.
[68]. Murphy AA, Santanam N, Parthasarathy S. Endometriosis: a disease of oxidative stress? Semin Reprod Endocrinol 16, 1998, 263-73.
[69]. Strandell A, Lindhard A. Why does hydrosalpinx reduce fertility? The importance of hydrosalpinx fluid. Hum Reprod 17, 2002, 1141-5.
[70]. Bedaiwy MA, Goldberg JM, Falcone T, Singh M, Nelson D, Azab H, et al. Relationship between oxidative stress and embryotoxicity of hydrosalpingeal fluid. Hum Reprod 17, 2002, 601-4.
[71]. Nasr-Esfahani MH, Winston NJ, Johnson MH. 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, 1992, 219-31.

[72]. Goto Y, Noda Y, Mori T, Nakano M. Increased generation of reactive oxygen species in embryos cultured in vitro. Free Radic Biol Med 15, 1993, 69-75.
[73]. Yang HW, Hwang KJ, Kwon HC, Kim HS, Choi KW, Oh KS. Detection of reactive oxygen species (ROS) and apoptosis in human fragmented embryos. Hum Reprod 13, 1998, 998-1002.
[74]. Guyader-Joly C, Guerin P, Renard JP, Guillaud J, Ponchon S, Menezo Y. Precursors of taurine in female genital tract: effects on developmental capacity of bovine embryo produced in vitro. Amino Acids 15, 1998, 27-42.
[75]. Paszkowski T, Clarke RN. The Graafian follicle is a site of L-ascorbate accumulation. J Assist Reprod Genet 16, 1999, 41-5.
[76]. Bedaiwy MA, Falcone T, Al-Hussaini T, Abdel-Aleem A, Sharma RK, Worley SE, et al. Differential growth of human embryos in vitro: role of reactive oxygen species. Fertil Steril 82, 2004, 593-600
[77]. Sukcharoen N, Keith J, Irvine DS, Aitken RJ. Predicting the fertilizing potential of human sperm suspensions in vitro: importance of sperm morphology and leukocyte contamination. Fertil Steril 63, 1995, 1293-300.
[78]. Zorn B, Vidmar G, Meden-Vrtovec H. Seminal reactive oxygen species as predictors of fertilization, embryo quality and pregnancy rates after conventional in vitro fertilization and intracytoplasmic sperm injection. Int J Androl 26, 2003, 279-85.
[79]. Alvarez JG. Nurture vs nature: how can we optimize sperm quality? J Androl 24, 2003, 640-8.
[80]. Agarwal A, Nallella KP, Allamaneni SSR, Said TM. Role of antioxidants in treatment of male infertility: an overview of the literature. Reprod Biomed Online 8, 2004, 616-27.
[81]. Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. J Androl 17, 1996, 530-7.
[82]. Vezina D, Mauffette F, Roberts KD, Bleau G. Seleniumvitamin E supplementation in infertile men. Effects on semen parameters and micronutrient levels and distribution. Biol Trace Elem Res 53, 1996, 65-83.
[83]. Lenzi A, Picardo M, Gandini L, Lombardo F, Terminali O, Passi S, et al. Glutathione treatment of dyspermia: effect on the lipoperoxidation process. Hum Reprod 9, 1994, 2044-50.
[84]. Comhaire FH, Christophe AB, Zalata AA, Dhooge WS, Mahmoud AM, Depuydt CE. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostaglandins Leukot Essent Fatty Acids 63, 2000, 159-65.
[85]. Vicari E, Calogero AE. Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis. Hum Reprod 16, 2001, 2338-42.
[86]. Vicari E, La Vignera S, Calogero AE. Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculoepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. Fertil Steril 78, 2002, 1203-8.
[87]. Lenzi A, Lombardo F, Sgro P, Salacone P, Caponecchia L, Dondero F, et al. Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertil Steril 79, 2003, 292-300.
[88]. Lewin A, Lavon H. The effect of coenzyme Q10 on sperm motility and function. Mol Aspects Med 18, 1997, Suppl, S213-9.
[89]. Henmi H, Endo T, Kitajima Y, Manase K, Hata H, Kudo R. Effects of ascorbic acid supplementation on serum progesterone levels in patients with a luteal phase defect. Fertil Steril 80, 2003, 459-61.
[90]. Crha I, Hruba D, Ventruba P, Fiala J, Totusek J, Visnova H. Ascorbic acid and infertility treatment. Cent Eur J Public Health 11, 2003, 63-7.
[91]. Catt JW, Henman M. Toxic effects of oxygen on human embryo development. Hum Reprod 15(2), 2000, 199-206.
[92]. Wang X, Falcone T, Attaran M, Goldberg JM, Agarwal A, Sharma RK. Vitamin C and vitamin E supplementation reduce oxidative stress-induced embryo toxicity and improve the blastocyst development rate. Fertil Steril 78, 2002, 1272-7.