



Male Fertility

Role of oxidant stress and antioxidant therapy

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Infertility is a significant problem affecting 15% of couples worldwide. Forty percent (40%) of infertility cases are attributable to male factors. Chronic and excessive levels of oxidative stress are increasingly recognized to contribute to chronic disease development, and a growing body of evidence points to a significant role of oxidative stress in the pathogenesis of infertility in men. This article outlines the pathogenic role of oxidative stress in male infertility and provides an overview of the evidence supporting the therapeutic use of selected dietary antioxidants (L-carnitine and L-acetyl-carnitine, selenium, N-acetyl-cysteine, folate, zinc and vitamin C) in the integrative care of men with this condition.

Male Factor Infertility

Male factor infertility accounts for 40% of all cases of infertility (Akmal 2006, Tremellen 2008). The most common form of male infertility is oligoasthenoteratospermia (OAT) (Cavallini 2006, Ross 2010). There is no accepted medical treatment for OAT, while mechanical techniques such as IVF-ICSI (in vitro fertilization/intra-cytoplasmic sperm injection) are used to circumvent it (Cavallini 2006, Ross 2010, Safarinejad 2009). However, these procedures fail to address the potentially reversible, underlying causes of OAT (Ross 2010) and are not without significant physical, emotional and financial consequences for couples.

While thirty percent (30%) of OAT cases are diagnosed as idiopathic (Cavallini 2006, Ross 2010), several factors including subtle endocrine abnormalities, environmental toxins, chronic inflammation or infection, obesity, sperm autoimmunity, genetic or constitutional factors, and excessive levels of oxidative stress induced by reactive oxygen species (ROS), have been implicated in the pathogenesis of OAT (Cavallini 2006, Comhaire 2000, Ross 2010, Safarinejad 2009). Many of the aforementioned factors can independently increase oxidative stress, while additional oxidation resulting from psychological stress, chronic diseases such as diabetes, smoking, alcohol, certain drugs, low fruit and vegetable intake may further contribute to OAT (Tremellen 2008). In fact, up to 30% to 80% of cases of male infertility have been linked to oxidative stress (Tremellen 2008 citing McLachlan 2001).

Oxidative Stress and Male Infertility

Seminal plasma contains the highest concentration of antioxidants of any human fluid (Cavallini 2006). In fact, sperm and seminal plasma are natural repositories for enzymatic and non-enzymatic antioxidants, including superoxide dismutase, glutathione peroxidase, catalase, vitamins C and E, glutathione and carnitine (Ross 2010), which are capable of protecting sperm from ROS-induced damage (Cavallini 2006). The principal generators of ROS in seminal fluid are sperm cytoplasm and leukocytes (Ross 2010).

Spermatozoa produce ROS as part of normal metabolic processes, and are particularly vulnerable to oxidative stress due to their high cell membrane polyunsaturated

fatty acid content, limited capacity for DNA repair, and the removal of most of their antioxidant-containing cytoplasm during sperm maturation (Ebisch 2007, Menezo 2007).

Despite the normally high seminal antioxidant content, excessive generation and/or decreased scavenging of ROS may lead to accumulation of ROS with consequent oxidative damage to sperm DNA, cell membranes and proteins, leading to apoptosis (and thereby oligospermia), atypical morphology (teratospermia) and impaired motility (asthenospermia) (Ebisch 2007, Ross 2010). Oxidative cell membrane damage also impairs sperm-oocyte fusogenic capacity (Tremellen 2008). Oxidative DNA damage, including that induced through certain assisted reproductive technologies, can compromise the paternal genomic contribution to the embryo and could result in decreased pregnancy rates, increased miscarriage risk and unknown genetic consequences in offspring (Comhaire 2003, Menezo 2007, Tremellen 2008).

ANTIOXIDANT THERAPY IN INFERTILITY: SELECTED AGENTS

Carnitine

In a review of prospective clinical trials, Cavallini et al. (2006) found L-carnitine (LC) and L-acetyl-carnitine (LAC) to be significantly more effective than placebo at improving fertility in men with idiopathic OAT. Zhou et al. (2007) systematically reviewed nine randomized controlled trials involving a total of 862 infertile men aged 18-65 years, the majority diagnosed with OAT, who had taken either LC, LAC, both carnitines together, or carnitines with other agents (NSAIDs, vitamin E or vitamin C) at dosages of 2-3 g daily in single or divided doses for 24 weeks to six months (Zhou 2007). Meta-analysis of seven eligible trials identified a markedly significant increase in pregnancy rates with carnitines, reporting 55 pregnancies in the treatment group and nine in the control group (odds ratio=4.10, 95% CI: (2.08, 8.08)), ($p<0.0001$). Compared to placebo or other agents, carnitines also significantly increased total sperm motility ($p=0.01$) and forward motility ($p=0.04$) and decreased atypical morphology ($p<0.00001$), but did not significantly influence sperm concentration.

LC is concentrated by active transport from the systemic circulation into the epididymal lumen (Ahmed 2011) where it accumulates as both LC and LAC (Zhou 2007). During sperm maturation in the epididymis, spermatozoa themselves accumulate LC (Ahmed 2011). LC acts as an antioxidant in seminal fluid, protecting sperm membranes and DNA from various mechanisms of oxidative stress (Zhou 2007). LC is essential to mitochondrial beta-oxidation of long chain free fatty acids (Zhou 2007, Moradi 2010), removes excess intracellular toxic acetyl-CoA (Zhou 2007) and is believed to serve as a post-ejaculatory energy source for sperm (Ahmed 2011).

Ahmed et al. (2011) found seminal LC levels to be significantly lower in infertile men than

sperm motility and function, selenium (Se) is considered to improve semen quality and male fertility through the action of glutathione peroxidases (GPXs), Se-containing antioxidant enzymes in seminal fluid that decrease propagation of ROS by reducing H₂O₂ and lipid peroxides to alcohols and water (Cavallini 2006, Mistry 2012, Safarinejad 2009). The intracellular antioxidant glutathione (GSH) is a cofactor for GPX and reacts directly with ROS and cytotoxic aldehydes to protect sperm from the effects of lipid peroxidation (Atig 2012). N-acetyl-cysteine (NAC) is a derivative of L-cysteine and a precursor to GSH that also possesses direct free radical scavenging activity (Safarinejad 2009). An open prospective study involving 27 infertile men showed that 600 mg NAC plus fatty acid supplementation (1 g docosahexaenoic acid, 0.25 g gamma-linolenic acid and 0.10 g arachidonic acid) daily significantly reduced seminal ROS and increased sperm count in oligospermic men (Comhaire 2000). A recent prospective controlled study involving 250 infertile men identified significant positive correlations between seminal selenium and selenoenzyme concentrations and sperm motility (Atig 2012).

Investigating the effects of Se, NAC, or the two antioxidants in combination, Safarinejad and Safarinejad (2009) conducted a double-blind, placebo controlled, randomized trial in a cohort of 468 infertile men with idiopathic OAT. Participants received either 200 µg Se (n=116), 600 mg NAC (n=118), 200 µg Se plus 600 mg NAC (n=116), or placebo (n=118) daily for 26 weeks. None of the participants had Se deficiency or low blood plasma NAC at the outset of the study. Compared to placebo, all three treatment groups showed significant improvements from baseline in sperm concentration, mean total sperm count (30% increase), motility (19% increase), and normal morphology (26% increase). Se and/or NAC also produced significant

“Seminal plasma contains the highest concentration of antioxidants of any human fluid”

fertile men, while higher seminal carnitine levels have been positively associated with improved sperm count, motility and normal morphology. Comparing the effects on sperm parameters of LC (2 g/day) to those of the anti estrogen, clomiphene citrate (25 mg/day), Moradi et al. (2010) found LC to be superior to clomiphene at increasing semen volume and equally effective at increasing sperm count and motility after three months of therapy; LC induced no significant effect on sperm morphology (Moradi 2010).

Selenium and N-acetyl-cysteine

Essential to normal spermatogenesis,

decreases in serum LH and FSH and increases in serum testosterone and inhibin B, a marker of Sertoli cell function (Cavallini 2006). Seminal plasma Se and NAC were significantly and positively correlated to sperm count, concentration, motility and percent normal morphology, with the sum effects of seminal Se and NAC showing stronger correlations with increased sperm concentration ($r=0.67$, $p=0.01$), motility ($r=0.64$, $p=0.01$) and percent normal morphology ($r=0.66$, $p=0.01$). No adverse events were reported with Se or NAC intake (Safarinejad 2009).

Folate and Zinc

Zinc (Zn) is concentrated in the prostate gland, seminal plasma and spermatozoa (Atig 2012, Ebisch 2007). It is a cofactor for more than 80 metalloenzymes involved in DNA transcription, translation and repair, underscoring its importance in sperm cell development (Atig 2012, Colagar 2009, Ebisch 2007). Zn has important antioxidant and anti-apoptotic properties and supports the function of steroid hormone receptors (Atig 2012, Ebisch 2007). Depleted seminal plasma Zn levels have been correlated with idiopathic subfertility and lower sperm counts in several studies (Ebisch 2007). Significant and positive correlations have been reported between seminal Zn levels and sperm count ($r=0.32$, $p<0.01$) and normal morphology ($r=0.42$, $p<0.001$), with significantly higher seminal Zn levels noted among fertile men than infertile men (Colagar 2009). A recent prospective trial of 250 infertile men found seminal Zn concentrations to be significantly and positively correlated with sperm motility and concentration (Atig 2012). Based on National Health and Nutrition Examination Survey (NHANES, 1999-2000) (U.S.) data, 79% of men are estimated to consume less than the recommended dietary allowance (RDA) of Zn in their diets (Young 2008).

Zn is also a cofactor for the folate-metabolizing enzymes dihydrofolate reductase and gamma-glutamyl hydrolase, as well as for methionine synthetase and betaine-homocysteine methyltransferase, which indirectly support the folate cycle through methionine metabolism (Ebisch 2007). Both folate and zinc possess anti-apoptotic properties, although excessively high zinc levels can induce apoptosis and necrosis (Ebisch 2007). Zinc deficiency decreases the intestinal absorption and

metabolism of folate (Ebisch 2007, Forges 2007).

Folate is essential to healthy reproduction due to its role in nucleic acid synthesis and thereby the proliferation of rapidly dividing cells including sperm cell precursors (Ebisch 2007, Forges 2007). Folic acid, the synthetic form of folate, scavenges free radicals and prevents lipid peroxidation in sperm cell membranes and protects DNA from oxidative damage (Ebisch 2007). Various folates are concentrated in seminal plasma and the concentration of non-methyltetrahydrofolate has been significantly positively correlated with sperm count and concentration (Forges 2007). Folate may have a beneficial effect on spermatogenesis through improving cohesion of seminiferous epithelial cells and thereby preventing premature release of immature sperm into the tubules (Forges 2007). Folate deficiency-induced hypomethylation of DNA and phospholipids may impair testicular exocrine and endocrine functions (Forges 2007). Folate deficiency also precipitates hyperhomocysteinemia, a pro-inflammatory state that is linked with poor sperm quality and male infertility (Forges 2007). Used in combination with other antioxidants, folic acid has improved sperm concentration and pregnancy rates in studies involving assisted contraception (Ross 2010 citing Tremellen 2007 and Wong 2002). NHANES data estimate that 64% of men consume less than the RDA of dietary folate (Young 2008).

Dividing 94 subfertile (sperm concentration 5×10^6 /ml to 20×10^6 /ml) and 99 fertile men into four treatment groups, folic acid (5 mg/day) and zinc sulfate (66 mg/day) together, folic acid or zinc plus a placebo, or two placebos, were administered to participants for six months (Forges 2007 citing Wong 2002). Folic acid and zinc together produced a substantial 74% increase in sperm concentration and count in subfertile men compared to fertile controls, although none of the men had zinc or folate deficiency prior to treatment (Forges 2007 and Comhaire 2000 citing Wong 2002). No significant changes were observed with separate administration of folate or zinc (Forges 2007 citing Wong 2002).

Vitamin C

Higher dietary and supplemental intake of vitamin C has been correlated with higher sperm count, concentration and progressive motility (Eskenazi 2005).

In a small open study involving 13 infertile men 25 to 35 years of age, 1000 mg vitamin C taken twice daily for two months produced significant increases in mean sperm count from 14.3 x 10⁶ to 32.8 x 10⁶ sperm/mL (p<0.001), normal morphology from 43% to 66.7% (p<0.001), and sperm motility from 31.2% to 60.1% (p<0.001) (Akmal 2006). A group of 36 infertile men with asthenoteratozoospermia and leukocytospermia showed significant improvements in progressive motility and morphology with decreased necrosis and leukocytosis after treatment with 60 mg vitamin C, 10 mg vitamin E, 100 mg fermented papaya, 194 mg lactoferrin and 40 mg beta-glucan daily for three months (Piomboni 2008). Ménézo (2007) administered a combination of 400 mg vitamin C with 400 mg vitamin E, 18 mg beta-carotene, 500 µmol Zn and 1 µmol Se daily for 90 days to 58 men with sperm DNA abnormalities and a history of at least two IVF or ICSI failures. Treatment

significantly decreased sperm DNA fragmentation, but paradoxically increased sperm head decondensation, a phenomenon that when exceeding a critical threshold of 28% is associated with greater risk of chromosome condensation abnormalities and poorer prognosis for pregnancy with IVF or ICSI (Ménézo 2007).

Conclusion

The majority of literature reviewed consists of relatively small but positive studies showing benefits to sperm quality and in some cases pregnancy rates with treatment of infertile men with various single or combination antioxidants, with few adverse effects. Recognizing the role of oxidative stress in the pathogenesis of numerous serious chronic diseases, reducing the oxidative burden in affected men is likely to benefit sperm quality and fertility as well as their overall health in the longer term. ■

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