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Antioxidant Supplementation and Duration of Antioxidant in Male Infertility – A Systemic Review

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Abstract*—Infertility, a global health issue affecting 187 million couples, is primarily caused by oxidative stress, particularly in males. High levels of reactive oxygen species (ROS) impair sperm quality, leading to oxidation of DNA, proteins, and lipids. Multi-antioxidant supplementation is effective in improving male fertility parameters. Natural molecules like myo-inositol and d-chiro-inositol also improve sperm quality. This review explores these processes and their mechanisms. Antioxidant treatment may improve sperm DNA fragmentation index (DFI) values in idiopathic infertility cases, prolonging treatment duration may enhance success, but side effects, cost, patient compliance, and partner condition should be considered. Antioxidant supplementation (AS) positively impacts male fertility, with beneficial ingredients like carnitines, Vitamin E, and zinc. Environmental factors may also influence fertility, requiring further studies to determine optimal combinations.*

***Keywords*—** *Antioxidant; male fertility; DNA fragmentation index.*

I. INTRODUCTION

Infertility is a global issue affecting over 80 million couples, with male infertility occurring in 30-50% of cases. Factors contributing to male infertility include varicocele, smoking, radiation, urinary tract infections, nutritional deficiencies, oxidative stress, and environmental factors. Oxidative stress, particularly reactive oxygen species (ROS), may play a role in male infertility (15). The Mediterranean diet has been shown to protect against male infertility and cancer risk. Sperm function tests and oral ascorbic acid (AS) have shown a positive correlation between AS and male fertility, but conflicting recommendations remain. Multi-antioxidant supplementation improves male fertility by decreasing ROS concentration and improving sperm quality through the synergistic effects of antioxidants, myo-inositol, and d-chiro-inositol (2).

Male factor reduction in reproductive potential accounts for 20%-70% of all infertility cases, emphasizing the importance of evaluating the male partner in infertility. The initial evaluation should include a medical and reproductive history and detailed examination, including a conventional semen analysis (14). Semen analysis is not only diagnostic but also aids decision-making in treatment, but it has methodological challenges despite being standardized by the World Health Organization (1).

Conventional semen analysis is insufficient to evaluate all cases of male infertility as it cannot fully assess functional adequacy. Studies on DNA integrity and spermatozoa fragmentation have been conducted to address this issue. Increases in sperm DNA fragmentation (SDF) are linked to recurrent pregnancy losses and negatively impact pregnancy and live birth rates in both natural and assisted reproductive procedures (16). Oxidative stress (OS) is one of the important causes of SDF, as it impairs the functions of spermatozoa and directly affects DNA integrity, quality, and function. Lifestyle changes and antioxidants can improve sperm quality by reducing the risk of SDF (17). However, the data necessary to adequately support these treatments are not yet available, leading to the need for standard treatment protocols and durations (3).

II. METHODS

The study reviewed the records of 637 patients who received antioxidant treatment due to increased sperm DNA damage between 2014 and 2019. The patients were evaluated through a comprehensive history and physical examination. After 2-7 days of sexual abstinence, they attempted to provide sperm samples at an assisted reproductive center's embryology laboratory using audiovisual stimulation. Semen samples were collected and analyzed using WHO criteria. Sperm DNA fragmentation index (DFI) was measured using TUNEL method. The study included 53 patients with a sperm DFI of 30% and above. Blood samples were taken for hormonal evaluation. Varicocele were excluded from the study due to its impact on spermatogenesis. The study used SPSS software for statistical analysis. The Shapiro-Wilk test was used to test for normal distribution of sperm DNA damage before and after treatment. The Friedman test was used to compare quantitative data in more than two dependent groups. A value of $p<0.05$ was considered statistically significant (3)

III. RESULTS

The study involved 53 patients with a mean age of 34.4 years, none of whom had concomitant health problems. The patients had a history of varicocelectomy, smoking, and alcohol use. Semen parameters were not significantly different between pretreatment and third and sixth months. However, the median DFI value decreased significantly between pretreatment and third and sixth months of antioxidant treatment. The DFI values decreased significantly between pretreatment and third months, and between the third and sixth months of antioxidant therapy. No side effects required discontinuation of antioxidant therapy in any patient (3)

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TABLE 1. Results of antioxidant treatment of third and sixth month (3)

Semen parameters	Pretreatment	Third month	Sixth month	p
Volume (mL)	3.23 (±1.55)	3.87 (±2.12)	3.9 (±1.95)	>0.05
Concentration (million/mL)	72.02 (±71.96)	70.06 (±37.71)	75.5 (±50.97)	>0.05
Total progressive Motility	47.41 (±25.02)	47.64 (±23.55)	48.3 (±26.3)	>0.05
Sperm DFI (median–IQR)	44% (13.7%)	33.3% (20.9)	18% (13.4%)	<0.001
DFI: DNA fragmentation index; IQR: Interquartile range				

IV. DISCUSSION

Sperm DNA damage (SDF) is a common issue in men with oligospermia, which can lead to impaired sperm functions and infertility (19). Factors such as age, poor lifestyle, environmental radiation exposure, concomitant diseases, medications, external genital tract infections, and varicocele can cause DNA breaks through OS, disrupting chromatin maturation, and apoptosis (28) The antioxidant system, which includes enzymatic factors and nutrients like selenium, zinc, and copper, neutralizes free radicals, leading to decreased antioxidant activity (18).

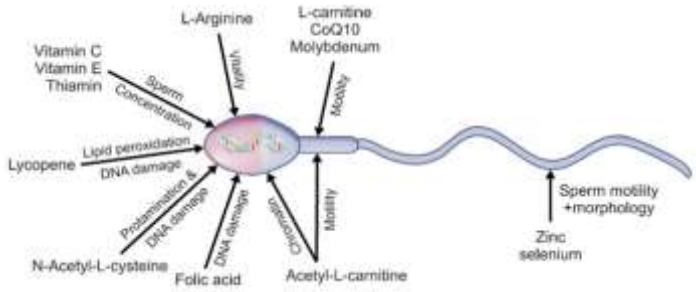

Fig. 1. Antioxidant site of action

Antioxidant therapy has been shown to prevent OS-related damage and improve SDF in infertile men. Many oral antioxidant preparations contain trace elements, and studies have shown improvement in at least one sperm parameter (20). However, some studies show no positive contribution to treatment success. The selection, dosage, and duration of antioxidant use are unclear, and it is recommended to take them in higher doses when OS is present (21).

In 34 randomized controlled trials using various antioxidant agents and a meta-analysis involving 2,876 couples, it was reported that antioxidant treatment has a positive effect on Long-term Reproductive Rate (LBR) and pregnancy rates in assisted reproductive methods (22). However, the duration of treatment has a significant variation. In this study, the DFI of subfertile men with oligospermia was improved in the third and sixth months, and extending the treatment to six months increased the positive effect on DNA damage (23). However, caution should be exercised when deciding whether to extend the duration of treatment (3).

SDF analysis tests have limitations, including methodological difficulties and lack of clinical reproductive outcomes. Larger-scale, randomized +- studies should be conducted to better understand the impact of antioxidant therapy on sperm DNA damage and improve treatment outcomes (24)

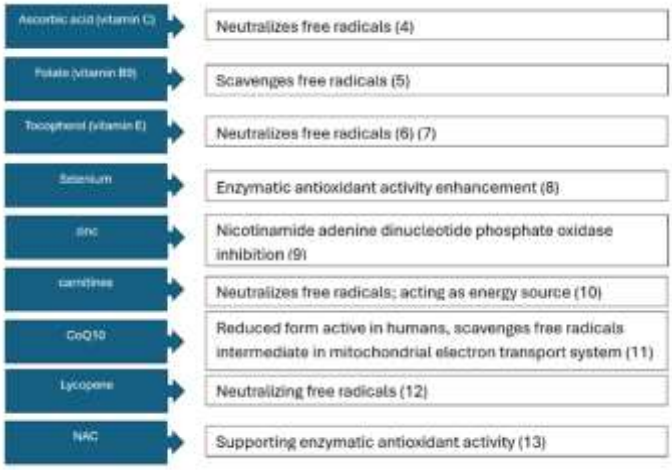

Fig. 2. Antioxidant mechanism of action

Ascorbic acid (vitamin C) is a water-soluble antioxidant that plays a crucial role in hydroxylation and amidation reactions. It is found in large amounts in seminal plasma and may prohibit DNA damage (25). Vitamin E is a fat-soluble antioxidant that neutralizes free radicals and protects cell membranes from free radicals. Studies have shown that higher doses of vitamin C and vitamin E can reduce DNA damage in infertile men (30). L-carnitine (LC) is essential for intermediary metabolism and plays a role in the formation of acyl carnitine esters. Studies have shown a positive association between LC and LAC and sperm motility (26). CoQ10 (ubiquinon) is another AS involved in aerobic cellular respiration and has been shown to improve sperm motility, density, and morphology. However, supplementation of CoQ10 in infertile men does not increase live-birth or pregnancy rates, but it shows general improvement in sperm parameters (27).

Zinc, a crucial metal in the body, is found in red meat, fish, and milk. Supplementation can protect spermatozoa against oxidized thiol levels, potentially restoring impaired semen function. Low zinc concentrations are associated with reduced fertilization capacity.

Selenium is essential for testosterone biosynthesis and sperm formation, and over 25 selenoproteins have been identified in humans. N-acetylcysteine, a naturally occurring compound, has been shown to improve sperm count, motility, morphology, and concentration in infertile men. Multi-Acrylonitrile (AS) therapy has shown effective results in male infertility, but the evidence is inconsistent. Studies have shown that AS treatment improves sperm quality, including basic semen parameters and DNA damage, and helps ensure DNA integrity and reduce sperm DNA fragmentation. However, there is no strong evidence of benefit from AS treatment, and extensive AS can lead to excess reductants and

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impede essential oxidation mechanisms, negatively affecting fertility (28).

Folate, a vitamin involved in DNA synthesis and oxidative pathways, is an antioxidant. It effectively scavenges oxidizing free radicals and inhibits LPO. Folate deficiency can increase lipid peroxidation indexes in cells. Supplementation with folic acid has been shown to improve sperm concentration and endocrine parameters in sub-fertile males. Folic acid may also stimulate Sertoli cells, a marker of good spermatogenesis (1).

L-carnitine, found in epididymal tissue, seminal plasma, and spermatozoa, plays a crucial role in mitochondrial metabolism. It transports acetyl and acyl groups, essential for mitochondrial metabolism, and plays a role in energetic metabolism, enhancing sperm motility and maturation. L-carnitine also acts as a free radical scavenger, protecting against oxidative damage. Studies have shown that L-carnitine improves sperm vitality, motility, and reduces ROS, and can be combined with other treatments for improved fertility (1).

L-arginine plays a role in sperm formation and prevents membrane lipid peroxidation through the production of nitric oxide (NO). In vitro studies show that low concentrations of NO increase sperm capacitation and enhance tyrosine phosphorylation in sperm proteins. L-arginine may be beneficial for artificial insemination in men with abnormal spermatozoa motility, as it enhances sperm metabolism and decreases membrane lipid peroxidation. Studies have shown that L-arginine improves sperm volume, concentration, motility, vitality, and morphology without adverse effects (1).

N-acetyl-cysteine (NAC) has been used as a mucolytic agent since the 1960s to break disulfide bonds in mucus glycoproteins, reducing viscosity. It is considered an option for treating diseases involving oxidative stress (29). NAC's antioxidant activity can be attributed to three mechanisms: direct antioxidant effect against certain oxidant species, indirect antioxidant effect through NAC acting as a precursor to cysteine, and chemically acting as a reducing agent. Animal models have also shown NAC's efficacy in various conditions, such as the protective effect against toxic effects of TiO2 nanoparticles, arsenic trioxide, and chlorpyrifos. In vitro studies have shown that NAC can improve sperm parameters, chromatin negative alteration, and hormonal profile. In vivo studies have also shown that NAC can improve protamine deficiency and DNA fragmentation (28).

V. Conclusion

Antioxidant therapy (AS) has been extensively studied for improving male fertility, with commonly used preparations including vitamin E, carnitines, vitamin C, CoQ10, NAC, zinc, folic acid, selenium, and lycopene. However, identifying an ideal treatment method remains challenging due to heterogeneity and factors. Oral antioxidants can reduce OS-related sperm DNA damage in male patients with idiopathic infertility associated with increased SDF. A 6-month treatment period may contribute to treatment success, but considering side effects, cost, patient compliance, and partner condition is crucial for treatment planning.

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