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Review Article

The Contribution of Antioxidant Treatment on Sperm Parameters, DNA Integrity and ICSI Results

Marwa Lahimer[1,2,3*], Oumaima Gherissi[1], Henda Mustapha[1], Samira Ibala[1], Hédi Khaïri[6], Hafida Khorsi Cauet[2,5], Moncef BenKhalifa[2,5], Habib Ben Ali[4], Mounir Ajina[1,3]

[1]Department of Reproductive Biology, University Hospital Farhat Hached, Sousse, Tunisia; [2]Department of Cardio-respiratory Physiotherapy, University of Picardy Jules Verne, Amiens, France; [3]Department of Physiology and Physiopathology, University Hospital Farhat Hached, Sousse, Tunisia; [4]Depatment of Medicine, University of Sousse, Sousse, Tunisia; [5]ART and Reproductive Biology laboratory, University Hospital and School of Medicine, CHU Sud, France; [6]Department of Gynecology and Obstetrics, University Hospital Farhat-Hached, Sousse, Tunisia

ABSTRACT

In the etiology of male infertility, Oxidative Stress (OS) is a major cause of semen decline. The association between OS and male infertility is well known, estimated to be present at elevated levels in as many as 30%-80% of men experiencing infertility. Spermatozoa are particularly susceptible to oxidative damage due to their high content of polyunsaturated fatty acids and limited capacity for antioxidant defense mechanisms. OS occurs when there is an imbalance between the production of Reactive Oxygen Species (ROS) and the body's ability to neutralize or repair their harmful effects. Accumulating evidence suggests that OS contributes to lipid peroxidation, protein oxidation, mitochondrial dysfunction, and genome decay. Antioxidant treatments play a crucial role in mitigating OS by scavenging free radicals and preventing cellular damage. Effective clinical decision-making requires a thorough examination of the efficacy of antioxidant components. It is crucial to thoroughly assess the effectiveness of these antioxidant components such as L-carnitine, L-glutathione, Coenzyme Q10, selenium, and zinc for enhancing male fertility. Ongoing research consistently supports the clinical efficacy and impact of each individual antioxidant component. The aim of this study is to review the contribution of antioxidant supplements on improving semen parameters such as sperm volume, motility, concentration, morphology, DNA integrity (including maturity and DNA fragmentation), and Assisted Reproductive Technology (ART) outcomes.

Keywords: Antioxidant; Oxidative stress; Male infertility; Semen quality; Genome decay; ART outcome

INTRODUCTION

Male infertility is defined as the inability to achieve a pregnancy after 12 months of regular unprotected intercourse. It has a global impact on about 48 million couples and roughly 186 million individuals, affecting around 20% of couples in their reproductive years [1-3]. In 1992, Carlsen and colleagues confirmed in a large meta-analysis that sperm counts had declined by 50% during a 50-year [4]. Numerous studies have shown similar declines in sperm parameter globally [5,6]. Understanding the etiology of male infertility is crucial for diagnosis, treatment, and prevention. It can involve various endogenous and exogenous factors. The endogenous factor includes genetic factors (Y Chromosome Microdeletions, genetic disorders, hormonal imbalances, varicocele advanced age, sperm infections and leucospermia [7-14]. On the other hand, the exogenous factor include environmental factors, lifestyle factors [15-19].

These factors can contribute to oxidative stress in the male reproductive system. It occurs when there is an imbalance between

Correspondence to: Marwa Lahimer, Department of Reproductive Biology, University Hospital Farhat Hached, Sousse, Tunisia, E-mail: Lahimermarwa3@gmail

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Reactive Oxygen Species (ROS) production and the body's ability to neutralize them with antioxidants. This imbalance detrimentally affects both the quality and function of sperm [20,21]. Over-production of ROS may explain the excessive structural defect of sperm DNA at the nuclear [22], and mitochondrial levels. Mitochondrial DNA (mtDNA) is particularly susceptible to ROS-induced damage due to its proximity to the electron transport chain, which generates reactive oxygen species as a byproduct of oxidative phosphorylation. In addition to causing direct damage to mtDNA, ROS can also deplete mtDNA by inducing apoptosis or inhibiting mtDNA replication, leading to mitochondrial dysfunction. Sawyer, and al, assessed the susceptibilities of DNA in the mitochondrial to the damaging effects of hydrogen peroxide in two murine germ cell lines, by using a Quantitative Polymerase Chain Reaction Assay (QPCR) to measure the damage in mtDNA. They found that mtDNA is highly susceptible to the damaging effects of hydrogen peroxide and suffered extensive damage. These finding demonstrate that mtDNA is a sensitive biomarker for oxidative stress in germ line cells of mouse [23]. Cells have evolved antioxidant defence systems, such as superoxide dismutase and catalase, which can neutralize reactive oxygen species and prevent oxidative damage to nuclear and mtDNA. In addition, spermatogenesis and sperm DNA integrity were shown to be affected by unsuitable vitamins intake [4]. Several studies reported that micronutrient treatment based on l carnitine and zinc have a positive effect on semen parameters and function, ART outcomes and live birth rates. A study published by Saya in 2019 involved 175 males aged between 19 and 44 years old with idiopathic oligoasthenozoospermia and failed to conceive for 12 months. They are treated with Proxeed plus during 3 months, the results show an improvements in sperm volume, progressive motility and vitality and a significant reduction in DNA fragmentation index [24].

Diets containing sufficient amounts of antioxidants and vitamins A, B, C, and E can protect sperm DNA from cellular oxidation and damage, improving sperm quality, couples' live birth rates which in turn raises the rates of males fertility [25-27]. Furthermore, Cavallini, et al., investigated the effect of medical therapy on the decline of sperm aneuploidy levels and the results of Intracytoplasmic Sperm Injection (ICSI) improvement in patients with severe idiopathic oligoasthenoteratospermia [28]. The patients treated for 3-month with L-carnitine 1 g and Acetyl-L-carnitine 500 mg given twice per day and 30 mg cinnoxicam tablet every 4 days. The finding shows the 3 month antioxidant treatment lead to a significant reducing of aneuploid sperm frequency associated with more favourable ICSI outcome by increasing of the number of biochemical/clinical pregnancies and live births [29]. Therefore, this mini-review study focusing on the contribution of antioxidant supplementation in reproductive health improvement including semen quality, DNA integrity and Intracytoplasmic sperm injection outcome.

LITERATURE REVIEW

Antioxidant treatment: New insight in infertility

Antioxidant treatment in male infertility is a subject of interest and research, with studies exploring the potential benefits of antioxidants in improving various aspects of reproductive health [21,30]. Growing evidence reported that different antioxidants may have varying roles in protecting cells and preserving fertility. It's necessary to investigate the role of each component. Yaris, et al., reported that the combinations of both antioxidants (l-carnitine-1 g, Acetyl-l-carnitine-0.5 g, fructose-1 g, citric acid-0.50 mg, selenium-50 µg, coenzyme Q10-20 mg, vitamin C-90 mg, zinc-10 mg, folic acid-200 µg, and vitamin B12-1.5 µg) and the second combination (l-carnitine-500 mg, selenium-50 µg, coenzyme Q10-20 mg, vitamin C-60 mg, zinc-15 mg, folic acid-400 µg, vitamin E, and ginseng-15 µg) for 6 months had a positive effect on sperm parameters [30]. Joao, et al., investigated the sperm static oxido-reduction potential using male infertility oxidative system, in this case–control study, they recruited 134 men with normal sperm parameters and 547 men with abnormal sperm parameter. 604 patients of population have assessed for DNA integrity. They found that the oxido-reduction potential ratio was significantly important in men with abnormal sperm parameter when compared to men with normal sperm parameter. Also they found the DNA integrity of men with abnormal sperm was significantly more affected by static oxido-reduction potential index [31].

Common antioxidants studied in the context of male fertility include vitamin C, vitamin E, coenzyme Q10, zinc, and selenium. These antioxidants can be obtained through a balanced diet or, in some cases, through supplementation. L-Carnitine is a naturally occurring compound and a dietary supplement that plays a crucial role in the production of energy by transporting fatty acids into cells' mitochondria, which act as engines within cells, burning these fats to create usable energy [32,33]. The study of Ma, et al., reported that L-carnitine improves sperm count, morphology, and sperm motility, while also improving testosterone and LH levels [34]. A network meta-analysis incorporating 23 randomized controlled trials comprising 1,917 patients and evaluating 10 different types of antioxidants demonstrated that L-Carnitine yielded the most favorable outcomes concerning sperm motility and morphology [35]. In addition, the study of Sicchieri, et al., aimed to assess the effectiveness of a synthetic cryoprotectant enhanced with L-α-phosphatidylcholine and L-acetyl-carnitine on the maintain of sperm motility and chromatin quality in cryopreserved semen samples. The study outcome revealed an improvement in motility characteristics [36].

Coenzyme Q10 or ubiquinone, is a naturally occurring compound found in the cells of the human body. It plays a crucial role in the production of energy within the mitochondria, the energy powerhouse of cells. Coenzyme Q10 is a is a cofactor and an antioxidant, it's a key component of the electron transport chain, which is responsible for generating ATP [35]. The study of Ahmadi, et al., demonstrated that Q10 improved sperm motility in patients with idiopathic OAT [37,38]. Similarly, the study of Cheng, et al., reported that the antioxidant treatment with L-carnitine and Q10 can improve the semen parameters and outcome of clinical pregnancy in the OAT patient [35]. Zinc represents the predominant element within human semen, with concentrations notably surpassing those in the bloodstream. This high zinc content in seminal plasma primarily stems from the prostate gland, serving as a reflection of its secretory activity. A deficiency in zinc can lead to impaired spermatogenesis, reduced testosterone levels, and overall compromised male reproductive health [39]. It is involved in antioxidant defense, storage, production, secretion, and function of several enzymes which play important roles in hormone regulation and meiosis during spermatogenesis [40].

A review published by Almujaydil, et al., reported that Vitamins including Vit C, Vit B12, Vit E and trace elements serve as nutritional regulators, effectively decreasing oxidative stress and consequently improving sperm quality. This improvement is closely linked to the enhanced functioning of sperm mitochondria [41]. In addition, accumulating evidence show that the administration of vitamin D in men experiencing sub-fertility has been shown to have a positive effect on semen quality. This is achieved through improvements in sperm motility, enhanced sperm function, and an overall enhancement of *in vitro* fertility competence [42,43]. Moreover, Selenium is an essential trace element that plays a crucial role in various physiological processes, including male fertility. It is a component of selenoproteins, which are important for maintaining sperm function and involved in the regulation of spermatogenesis, the process of sperm production. Selenoproteins, such as glutathione peroxidases, help protect sperm cells from oxidative stress, contributing to normal sperm development [44,45].

Effect of antioxidant treatment

Semen quality: Antioxidants have been investigated for their potential to improve sperm quality, including sperm concentration, motility, and morphology. Studies suggest that supplementation with antioxidants may help reduce oxidative stress-related damage to sperm cells and enhance their overall functionality. A double blind randomised clinical trial investigated the effect of antioxidant supplementation based on L carnitine on conventional sperm parameter, sperm DNA fragmentation, sperm maturityand pregnancy achievement. The study results reported a significant increase in sperm motility and a significant decrease in sperm fragmentation [46]. A network meta-analysis of Randomized Controlled Trials (RCTs) treated a total of 23 RCTs including 10 types of antioxidants revealed that antioxidant supplementation with L-Carnitine improve sperm motility and morphology, while Omega-3 fatty acids improve sperm concentration. Additionally, Coenzyme-Q10 treated both sperm motility and concentration [47]. Another study published by Yaris, et al., involved 122 patients with idiopathic infertility, revealed that the combinations of antioxidants l-carnitine, acetyl-l-carnitine, fructose, citric acid, selenium, coenzyme Q10, vitamin C, zinc and folic acid administered for a period of 6 months had a beneficial effect on sperm parameters [30].

Genome integrity: Excessive ROS-induced damage to DNA can result in mutations and potentially contribute to the development of various diseases, including cancer. The cell has defense mechanisms to neutralize ROS and repair damaged DNA. Abad et al., aimed to assess the impact of a 3-month oral antioxidant treatment on sperm DNA fragmentation in 20 infertile patients with asthenoteratozoospermia. The treatment consisted of l-Carnitine, vitamin C, coenzyme Q10, vitamin E, zinc, vitamin B9, selenium, and vitamin B12. The study results showed a significant improvement in DNA integrity. The proportion of highly DNA degraded sperm also decreased significantly [48]. A systematic review and meta-analysis published in 2021 found a correlation between sperm DNA damage and elevated risks of miscarriage, transmission of genetic diseases, and potential compromise in both embryonic and subsequent postnatal development [49]. Moreover, a study conducted by Jannatifar et al., revealed that supplementation with N-acetyl-cysteine and alpha-lipoic acid had positive effects on sperm DNA fragmentation in individuals with OAT [50]. Hence, the advancement of antioxidant therapy holds the potential to mitigate DNA damage caused by oxidative stress, playing a crucial role in sustaining spermatogenesis [22,51]. Another notable concern revolves around the repercussions of certain sperm DNA damage. Meta-analyses suggest that if such damage remains unaddressed, it could elevate the risk of miscarriage and contribute to the transmission of genetic diseases, thereby compromising both embryonic and subsequent post-natal development [52].

Assisted reproductive technology outcome: Several studies have investigated the advantages of synergizing different antioxidants to mitigate oxidative stress damage and improve the rates of successful fertilization and pregnancy while reducing the occurrence of genetic abnormalities in offspring. Furthermore, scientific evidence suggests that antioxidants possess the capacity to influence cellular signaling pathways crucial for embryo development and implantation. Lahimer, et al., reported that the 3-month antioxidant treatment improve the pregnancy rate and the life birth rate compared to the placebo treatment [46]. Several reviews have also determined a positive impact of antioxidant therapy on achieving clinical pregnancy, whether through spontaneous conception or assisted reproduction methods [53,54]. A comprehensive analysis conducted by Smits, et al., incorporated data from 11 studies and concluded that there was an observed rise in the clinical pregnancy rate associated with diverse antioxidant treatments. In essence, the reported outcomes are consistent with the broader body of evidence presented in the Cochrane review, indicating a positive association between antioxidant interventions and increased rates of clinical pregnancy [55].

DISCUSSION

PMDS is a rare condition only very recently discovered. Basic mechanisms by which it develops are robustly established but molecular findings regarding genetic mutations accountable for suppressing Müllerian structures regression are still limited, mainly due to the scarcity of cases. It seems mandatory that clinicians from different fields such as pediatricians, gynecologists, obstetricians, family doctors and general practitioners are able to identify and consider the possibility of PMDS in its often subtle presentations. Considering treatment, surgical approaches seem rather successful, while robot-assisted procedures may help establish advanced management protocols in coming years. A main concern rarely mentioned is to provide psychological support for patients. Furthermore, as medical imaging and information spreads, it is reasonable to expect an increase in incidence following detection improvement. Indeed, there has been ongoing research on the potential impact of antioxidants on male infertility, and the results are somewhat mixed. The effectiveness of antioxidant supplementation in improving fertility outcomes varies across studies. This variation depends on participant characteristics, study design, types of antioxidants studied and the dosage. More research is needed to better understand the optimal dosage, duration, and specific types of antioxidants [56].

Every person possesses a distinctive genetic composition, makes unique lifestyle choices, experiences distinct environmental exposures, and maintains an individual health status. These factors collectively impact how individuals respond to antioxidant supplementation. Variances in susceptibility to oxidative stress or differences in baseline antioxidant levels can influence the outcomes of supplementation. Therefore, personalized approaches that take into account these individual variations may be essential for maximizing the benefits of antioxidant interventions [57]. A study assessed by Nateghian, et al., investigated the protective impacts of Pentoxifylline (PT) and L-carnitine (LC). This study involved 26 samples derived from normozoospermic men, initially processed using the swim-up technique. These samples were divided into three groups: Untreated control, L-Carnitine (LC)-treated, and PT-treated, and then subjected to incubation for up to 12 days at 4°C-6°C. The results demonstrated that PT supplementation led to an increased percentage of motile spermatozoa compared to both control and LC-treated specimens. Conversely, LC supplementation resulted in a higher percentage of viable spermatozoa when compared to PT-treated and control samples. Throughout the 12-day storage period, the percentage of spermatozoa with normal protamine content remained relatively constant across all three groups [58]. A study involving 50 infertile men characterized by oxidative stress underwent a 3-month oral antioxidant treatment. The results indicated no significant effect in conventional sperm parameters such as concentration, motility, and morphology [59]. In addition, 48 infertile couples were given Fertimax2 antioxidant treatment for a minimum of two months. The results indicate that no notable distinctions in sperm parameters were detected between the group receiving treatment and the control group [60,61].

CONCLUSION

Antioxidants have been studied for their potential role in improving male fertility. It may contribute to the improvement of male fertility by reducing oxidative stress, enhancing semen parameters including sperm motility and morphology, protecting DNA integrity, and supporting reproductive health. We have to mention that individual responses to antioxidant supplementation may vary, and the underlying causes of male infertility can be diverse. Therefore, it's interesting to consult with a healthcare professional or a fertility specialist to identify the potential causes and recommend the appropriate interventions, which may include lifestyle changes, dietary modifications, or specific antioxidant supplementation tailored to the individual's needs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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