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ANDROLOGY

Sperm cyropreservation and oxidative damage. What does it mean?

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About 15% of couples that desire children are affected by infertility. Oxidative stress has been involved in unexplained subfertility with a male factor [1].

Although the excessive production of reactive oxygen species (ROS) is detrimental to human spermatozoa, there is a growing body of evidence that suggests that ROS are also involved in the physiological control of some sperm functions. Under physiological conditions, small amounts of ROS are produced by spermatozoids. These ROS are necessary for efficiency, acrosomal reaction, and finally fertilization [2], but its excessive levels can negatively affect sperm quality. There is a current presumption that the most prolific source of ROS in sperm suspensions is an NADPH oxidase located in leukocytes or in spermatozoa that produces superoxide, which is further converted to peroxide by the action of superoxide dismutase (SOD).

Since antioxidants suppress the action of ROS, these compounds have been used in the medical treatment of male infertility (they are beneficial in terms of improving sperm function and DNA integrity) or have been added to the culture medium during sperm separation techniques. Antioxidants have demonstrated their impact on sperm improvement in several studies, in particular in men with high levels of ROS in their sperm. However, in many cases, no beneficial effect was obtained after antioxidant supplementation. Negative effects could be observed in long–term treatment or with excessive doses. This medical therapy should not be used in patients with known genetic factors such as karyotype anomalies or Y chromosome deletion. Therefore, it is essential to perform a complete diagnostic workup of the man before deciding which men will respond to medical therapy and which will need to be referred to assisted reproduction.

Treatment of oxidative stress should first involve strategies to reduce or eliminate stress–provoking conditions including smoking, varicocele (increases ROS levels in testes and semen), genital infection, gonadotoxins, and hyperthermia. In recent years, interest has increased in the role of antioxidants and B vitamins as modulators of fertility outcome. The antioxidants – alpha–tocopherol (vitamin E), ascorbic acid (vitamin C), and the retinoids (vitamin A) – are potent scavengers of ROS. Deficient vitamin B concentrations cause elevated homocysteine concentrations and impair the remethylation cycle of phospholipids, proteins, DNA, and RNA. These processes are essential in spermatogenesis.

Treatment with oral antioxidants has generally been associated with improvement in sperm DNA integrity and in some cases pregnancy rates after assisted reproduction. Actually, antioxidants are provided in diet or can be found in enriched food. It is possible that a subset of infertile men with specific lifestyles (e.g., smoking, increased alcohol intake, and dieting) may be at risk for antioxidant deficiency, particularly vitamin C deficiency. A low intake of antioxidant nutrients was associated with a poor semen quality [3]. Overall, the data published suggest that no single antioxidant is able to enhance the fertilization capability of infertile men, whereas a combination of them seems to provide a better approach [4].

Few studies have shown that the incidence of ROS caused DNA fragmentation in ejaculated spermatozoa can be reduced by oral antioxidant treatment. Oral antioxidant treatment appears to improve ICSI (intracytoplasmic sperm injection) outcomes in those patients with sperm DNA damage, in whom this treatment reduces the percentage of damaged spermatozoa [5]. It is not clear why some men responded to antioxidants by reducing the extent of sperm DNA fragmentation while others did not. Greco et al. [6] suggest that the increased percentage of DNA–damaged spermatozoa may be a sequela of different pathophysiological mechanisms in different patients and only some of these conditions may be responsive to antioxidant treatment. This would also explain the discrepancies in the literature concerning the clinical usefulness of antioxidants in the treatment of male infertility (reviewed in Agarwal et al., 2004) [7].

Sperm cryopreservation is a widely used procedure in the context of assisted reproductive techniques. Cryopreservation and thawing is a procedure that inflicts irreversible injury on human spermatozoa. One of the possible mechanisms involved in sperm cryoinjury is apoptosis upon exposure to oxidative stress. Cryopreservation can also result in increased lipid peroxidation in human spermatozoa [8] and has

been shown to reduce antioxidant defenses. Thus, the observed protective effect of vitamin E addition on post–thaw motility might be due to vitamin E suppression of lipid peroxidation via the sperm plasma membrane. The positive effect of vitamin E on motility is greater in semen samples from men over 40 years of age. The spermatozoa from older males had increased ROS and lipid peroxidation, suggesting a reduced capacity to cope with oxidative stress [9].

During *in vitro* fertilization the seminal plasma is removed during semen processing and the toxic oxygen metabolites (generated by immature spermatozoa and leukocytes) are able to attack spermatozoa without being protected by seminal plasma antioxidants. In addition, the detrimental effect of oxidative stress on sperm functional competence can be exaggerated by the *in vitro* sperm processing techniques (centrifugation and prolonged incubation) that usually precede assisted reproductive techniques. The addition of an antioxidant to the cryopreservation medium (vitamin E, both ascorbate and catalase) significantly reduces ROS concentrations in post–thaw spermatozoa [10].

Taking into account the pros and the cons of antioxidant treatment of male infertility, the potential advantages that it offers cannot be ignored.

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