World Journal of Urology (2019) 37:1029–1034
https://doi.org/10.1007/s00345-019-02656-3

TOPIC PAPER

Optimizing male fertility: oxidative stress and the use of antioxidants

Joao Paulo Cardoso[1,3] · Marcello Cocuzza[1,3] · Dean Elterman[2]

Received: 20 October 2018 / Accepted: 25 January 2019 / Published online: 4 February 2019

Abstract

Purpose Management of male infertility is always a difficult task, with pathophysiology and available treatments often poorly understood. The purpose of this review was to summarize current evidence regarding the use of protective agents against reactive oxygen species (ROS), such as antioxidants that may be useful in the treatment of male factor infertility.

Methods For this publication, a search of studies concerning oxidative stress, male infertility and antioxidant therapy was performed using the search engines ScienceDirect, OVID, PubMed and MEDLINE. Articles published in languages other than English were not considered.

Results An interest in the physiologic and pathologic effects of ROS has grown. Nevertheless, use of antioxidants is challenging, considering the balance between physiological ROS activity and detrimental oxidative stress level. Several studies have shown positive outcomes in terms of semen parameters, with others having failed to do so. Available evidence is still limited in pregnancy and live birth rates.

Conclusions Protective agents against ROS, such as antioxidants, may have positive effects on semen parameters in some patients, although a widespread indication is still restricted by practical aspects, including unknown physiological levels of ROS and controversy regarding different concentrations and combinations of drugs.

Keywords Male infertility · Antioxidants · Reactive oxygen species

Introduction

Infertility, as defined by failure in spontaneous pregnancy after 1 year of unprotected intercourse, may affect 8–15% of couples. A male factor is solely responsible for up to 20% of the cases and contributes to 30–50% of couples' infertility [1]. Modern assisted reproduction techniques overcome severe male factor infertility, although application of these methods in all infertile couples would definitely represent overtreatment.

✉ Joao Paulo Cardoso
jpgrecocardoso@gmail.com

1 Divisao de Andrologia e Reproducao Humana, Departamento de Urologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil

2 Division of Urology, Department of Surgery, University of Toronto, Toronto, ON, Canada

3 Departamento de Urologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Av. Dr. Enéas de Carvalho Aguiar, 255 7º andar-Sala 710F, Cerqueira César, São Paulo, SP CEP 05403-000, Brazil

Oxidative stress and male infertility have been previously correlated, with an imbalance between oxidation and reduction associated with negative effects on cells. Shifts of the body redox balance toward any of the oxidative stress or reductive stress sides are considered deleterious [2]. Ideally, a careful diagnostic workup is necessary before any treatment, for adequate and individual treatment selection.

This article reviews reactive oxygen species (ROS) sources and discusses its physiological and pathological effects. We also discuss the use of protective agents against ROS, such as antioxidants, that may be useful in the treatment of male factor infertility.

Reactive oxygen species and effects of seminal oxidative stress

ROS are compounds of oxygen containing free and unpaired electrons on their outer orbit. Among these compounds are the superoxide anion (O^{2-}), hydroxyl (OH^{-}) and hydrogen peroxide (H_2O_2), all highly reactive and capable of protein, lipid and nucleic acid damage [3, 4].

Concerning human reproduction, a balance typically exists between ROS production and the antioxidant scavenging system located in the male reproductive tract. Low physiological levels of ROS are essential in the regulation of sperm functions including sperm capacitation, the acrosome reaction and sperm–oocyte fusion on fertilization signaling [5]. Other physiologic functions, such as mitochondrial activity and endothelial cell proliferation, demand a low level of oxidative stress [6]. Usually, both intrinsic and extrinsic mechanisms act for a redox balance. Among intrinsic enzymatic oxidants are catalase, thiol peroxidases and superoxide dismutase, apart from non-enzymatic antioxidants such as gluthatione. Exogenous micronutrients are usually provided by a well-balanced intake of vitamins and trace elements such as vitamin A, vitamin C, vitamin E, L-carnitine, *N*-acetylcysteine, selenium and zinc.

Excessive production of ROS in the semen may overwhelm the antioxidant defense mechanisms and imbalance redox status. After release of germinal epithelium, spermatozoa are devoid of cytoplasm and its protective enzymes catalase and superoxide dismutase. Moreover, due to their high levels of polyunsaturated fatty acids, sperm plasma membranes are highly susceptible to undergoing a lipid peroxidation process [7]—the removal of electrons from the lipid membrane by ROS.

Apart from changes of membrane fluidity itself, peroxidation triggers a sequence of redox reactions that generates aldehydes as end products. These mutagenic and genotoxic alkylating agents are capable of inducing DNA damages and protein changes. Stem cells have highly effective proofreading and DNA repair. This results in a very low spontaneous mutation rates in the germ line [8]. Nevertheless, the ability of self-repair and apoptosis is lost after meiosis for gamete production, with extensive DNA damage ultimately resulting in poor embryo development, miscarriage and consequences on the fertility of the offspring through Y chromosome changes [9]. This is especially important in the context of assisted reproduction techniques, when the selection pressure on high fertility genes is bypassed.

Sperm motility loss has also been correlated with increased ROS and oxidative stress, although the exact pathophysiology of this process is still unknown. Hypothesis also rely on lipid peroxidation, direct effect on vital energy metabolism enzymes such as G6PD or interrelated biochemical events with decreased protein phosphorylation [10].

Immature spermatozoa and leucocytes in the seminal plasma are the main sources of endogenous ROS. Residual cytoplasm on immature spermatozoa contains greater amounts of cytoplasmic enzyme glucose-6-phosphate dehydrogenase, with higher mitochondrial metabolism generating higher amounts of ROS [11]. Peroxidase-positive leucocytes are able to produce ROS 1000-fold more than spermatozoa [12], and are usually present concomitantly with infectious and inflammatory conditions such as epididymitis and prostatitis.

Other endogenous and exogenous conditions favor oxidative stress: varicocele, metabolic syndromes, cigarette smoking, alcohol abuse, recreational drug abuse, combined with some of modern days factors such as environmental pollution, ionizing radiation and mobile phone use.

Antioxidant therapy

Antioxidants comprise a relatively accessible and inexpensive therapy that has been used in infertile patients. It is

Table 1 Dosing of the commonly prescribed antioxidants on recent publications

Antioxidant compound	Mechanism of action	Dose
Tocopherol (vitamin E)	Neutralizes ROS	180 mg–1 g
CoQ10	Energy production, pro-motility molecule. Inhibits superoxide formation	20 mg–300 mg
NAC	Direct effect through hydroxyl radicals scavenging and precursor of glutathione, a neutralizer of ROS	600 mg
Folic acid (vitamin B9)	ROS scavenging, nucleic acid synthesis	200 µ–5 mg
Ascorbic acid (vitamin C)	Neutralizes ROS	10 mg–1 g
Selenium	Enhanced enzymatic antioxidant activity	50 µg–200 µg
Zinc	Decreases production of hydrogen peroxide and hydroxyl radicals; DNA and RNA metabolism, signal transduction	66 mg; 400 mg–5 g
Lycopene	ROS quenching	2–6 mg
Carnitine	Scavenges ROS	LC: 2 g and LAC: 1 g

NAC N-acetyl-L-cysteine, *CoQ10* co-enzyme Q10, *LC* L-carnitine, *LAC* L-acetyl carnitine

Reference: Majzoub and Agarwal [6]

Dosing of the most commonly prescribed antioxidants is variable among recent publications. Majzoub et al. performed a systematic review of the compounds and indications regarding semen parameters, DNA fragmentation, oxidative stress, improving success rates of assisted reproductive techniques and live birth, as summarized in the table

Table 2 Main studies on antioxidants use and respective level of evidence

Authors	Year	Journal	*N*	Population	Follow-up	Outcomes	Antioxidant (s)	Level of evidence	
No improvement									
Hawkes et al. [15]	2009	*Journal of Andrology*	54	Normozoospermia	12 months	No improvement: motility or morphology	Selenium 300 µg/day	1b	Individual RCT
Greco et al. [16]	2005	*Journal of Andrology*	64	TUNEL assay > 15% of DNA fragmentation index (DFI)	2 months	No improvement: sperm concentration, motility or morphology (WHO 1999) Improvement DFI	Vitamin C + vitamin E 1000 mg each/day	1b	Individual RCT
Sigman et al. [17]	1999	*Fertility and Sterility*	21	Asthenozoospermia	6 months	No improvement: sperm concentration, motility or morphology (WHO 1999) 1 pregnancy in IVF group and 1 spontaneous in placebo group	2000 mg L-Carnitine + 2000 mg L-acetyl carnitine/day	1b	Individual RCT
Rolf et al. [18]	1999	*Human Reproduction*	31	Oligozoospermia ($> 7 \times 10^6$/mL) and/or asthenozoospermia (< 50% motility)	2 months	No improvement: sperm concentration, motility or morphology (WHO 1992) No pregnancy	Vitamin C + vitamin E 1000 mg + 600 mg/day	1b	Individual RCT
Kessopoulou et al. [19]	1995	*Fertility and Sterility*	30	Higher ROS levels on chemiluminescence assay	3 months	Improvement in vitro zona binding, no improvement in semen parameters	Vitamin E 600 mg/day	1b	Individual RCT
Moilanen et al. [20]	1993	*International Journal of Andrology*	13	Asthenozoospermia and/or oligozoospermia	3 months	No improvement: sperm concentration, motility or morphology (WHO 1987) No pregnancy	Vitamin E 100 mg/day	1b	Individual RCT
Improvement									
Busetto et al. [21]	2018	*Journal of Andrology*	94	Asthenozoospermia and/or oligozoospermia and/or teratozoospermia with and without clinical varicocele	6 months	Improvements in concentration, total sperm count, progressive and total motility for both varicocele- and non-varicocele-treated groups	1000 mg L-carnitine, 725 mg fumarate, 500 mg acetyl-L-carnitine, 1000 mg fructose, 20 mg CoQ10, 90 mg vitamin C, 10 mg zinc, 200 µg folic acid and 1.5 µg vitamin B12	1b	Individual RCT

Table 2 (continued)

Authors	Year	Journal	*N*	Population	Follow-up	Outcomes	Antioxidant (s)	Level of evidence	
Nadjarzadeh et al. [22]	2011	*Journal of Endocrinological Investigations*	47	Asthenozoospermia and oligozoospermia	3 months	Improvement in semen antioxidant capacity No improvement of semen parameters	Co-enzyme Q10 200 mg/day	1b	Individual RCT
Safarinejad et al. [23]	2009	*Journal of Urology*	548	History of infertility; $> 5 \times 10^6$/mL	14 months	Improvements in motility and concentration	200 g selenium/day, 600 mg *N*-acetylcysteine/day; 200 g selenium + 600 mg *N*-acetylcysteine/day	1b	Individual RCT
Akmal et al. [24]	2006	*Journal of Medicinal Food*	13	OZ	2 months	Improvements in total sperm count, motility and morphology (hemocytometry)	Vitamin C 2000 mg/day	2b	Individual cohort studies
Lenzi et al. [25]	2003	*Fertility and Sterility*	86	Concentration: 10–20 10^6/mL; total motility: 10–30%; forward motility: 15%; atypical forms: 70%	8 months	Improvement in sperm concentration and total and forward sperm motility	L-Carnitine 2 g/day	1b	Individual RCT
Wong et al. [26]	2002	*Fertility and Sterility*	211	History of infertility and sperm concentration 5–20 × 106/mL	26 weeks	Increase in total sperm count with combined therapy	Folic acid 5 mg/day; zinc 66 mg/day	1b	Individual RCT
Omu et al. [27]	1998	*European Journal of Obstetrics & Gynecology and Reproductive Biology*	100	Asthenozoospermia > 40% nonmotile	6 months	Improvement in sperm motility	Zinc 500 mg/day	1b	Individual RCT

SR systematic review, *RCT* randomized controlled trials

Level of evidence: https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-March-2009/

based on the property of chemical or biological compounds to neutralize or scavenge ROS or enhance enzymatic antioxidant activity, with the logical hypothesis that an increased antioxidant intake would treat and prevent male infertility.

Ideally, an effective treatment would be tailored to the patient's redox status. Limitation of this assumption is a lack of a widely accepted method to measure redox status, a precise normal redox level or acceptable cutoff values [13]. Therefore, commonly prescribed antioxidants have variable data among recent publications regarding dosing ranges and combinations.

Antioxidant therapy is also challenging when the balance between physiological ROS activity and detrimental oxidative stress level is considered. If antioxidants can reduce the pathological effect of ROS on one hand, on the other hand an overconsumption of such compounds could interfere with redox balance, in a process called "antioxidant paradox". Although cells usually work in a reduced state, a certain degree of oxidation is necessary. Protein synthesis and gene transcription require oxidation, as well as apoptosis, an important cellular mechanism and an oxidative process itself. Nevertheless, caspase enzymes enrolled in apoptosis can be deactivated by excessive oxidative status. Antioxidants could therefore shift the balance toward either suppression or facilitation of apoptosis [4].

Recently, Majzoub et al. [6] performed a systematic review of antioxidants and indications. The described compounds, in monotherapy or combination, are: vitamin C (500–1000 mg) vitamin E (400 mg), carnitines (500–1000 mg), NAC (600 mg), zinc (25–400 mg), lycopene (6–8 mg), folic acid (0.5 mg) and co-enzyme Q10 (100–300 mg). The mechanism of action and doses are summarized in Table 1.

Evidence in antioxidant therapy

Overall, evidence regarding antioxidant therapy reflects the heterogeneity of compounds, dosing and clinical applications. Henkel et al. [13]. performed an interesting review of antioxidants use in male infertility, with the main studies summarized in Table 2. The authors highlight trials with effects in semen parameters and other studies that failed to show positive outcomes. Although the reviewers present mostly randomized controlled trials, studies are widely variable regarding the type of antioxidants and combinations, population selection, follow-up and outcomes.

In terms of reproductive outcomes, a 2014 Cochrane review considered live birth rate as the primary end point. The main comparison was use of antioxidants versus placebo or no treatment. Secondary outcomes included clinical pregnancy, adverse effects, level of sperm DNA fragmentation, sperm motility and sperm concentration. In this meta-analysis, Showell et al. [14] summarize the main results of 48 trials in a population of 4179 subfertile men. For live birth, the quality of evidence was low (according to GRADE), in only four studies and a total of 277 participants, with a OR of 4.21 (2.08–8.51). Clinical pregnancy rate also showed a low quality of evidence and an OR of 3.43 (1.92–6.11) in seven studies with 522 patients. Considering adverse effects, samples were small to draw conclusions. Researchers were also unable to perform a meta-analysis on head to head antioxidants comparison for clinical pregnancy, live birth, adverse effects or semen parameters.

Take home message

Pathophysiology of male infertility is often a poorly understood process, increasing difficulty in patient management. Although studies have shown a potential improvement of male fertility, a widespread indication for antioxidants is still restricted by practical aspects, including unknown physiological levels of ROS and controversy regarding different concentrations and combinations of drugs. Overall, available studies with antioxidants show high heterogeneity in design, dosing ranges and outcomes. Limited data are available on adverse effects, and further trials are needed to define precise and optimal use of such substances.

Author contributions JPC was responsible for data collection, writing and editing the manuscript. MC was responsible for writing and editing the manuscript. DE was responsible for writing and editing the manuscripts.

Funding None.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

Ethical statements The authors declare no conflict of interest. There was no research involving human participants and/or animals.

References

1. Agarwal A et al (2015) A unique view on male infertility around the globe. Reprod Biol Endocrinol 13:37. https://doi.org/10.1186/s12958-015-0032-1
2. Castagné V et al (1999) An optimal redox status for the survival of axotomized ganglion cells in the developing retina. Neuroscience 93(1):313–320
3. Greabu M et al (2008) Oxygen, a paradoxical element? Rom J Intern Med 46(2):125–135
4. Halliwell B (2000) The antioxidant paradox. Lancet 355:1179–1180

5. Saleh RA, Agarwal A (2002) Oxidative stress and male infertility: from research bench to clinical practice. J Androl 23(6):737–752
6. Majzoub A, Agarwal A (2018) Systematic review of antioxidant types and doses in male infertility: benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. Arab J Urol 16(1):113–124
7. Agarwal A, Saleh RA, Bedaiwy MA (2003) Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 79(4):829–843
8. Aitken RJ, Koopman P, Lewis SE (2004) Seeds of concern. Nature 432(7013):48–52
9. Aitken RJ, Krausz C (2001) Oxidative stress, DNA damage and the Y chromosome. Reproduction 122(4):497–506
10. Agarwal A, Makker K, Sharma R (2008) Clinical relevance of oxidative stress in male factor infertility: an update. Am J Reprod Immunol 59:2–11
11. Koppers AJ et al (2008) Significance of mitochondrial reactive oxygen species in the generation of oxidative stress in spermatozoa. J Clin Endocrinol Metabol 93:3199–3207
12. Ford WCL, Whittington K, Williams AC (1997) Reactive oxygen species in human sperm suspensions: production by leukocytes and the generation of NADPH to protect sperm against their effects. Int J Androl 20(Suppl 3):44–49
13. Henkel R, Sandhu IS, Agarwal A (2019) The excessive use of antioxidant therapy: a possible cause of male infertility? Andrologia 51(1):e13162
14. Showell MG et al (2011) Antioxidants for male subfertility. Cochrane Database Syst Rev 19(1):CD007411. https://doi.org/10.1002/14651858.CD007411.pub2
15. Hawkes WC, Alkan Z, Wong K (2009) Selenium supplementation does not affect testicular selenium status or semen quality in North American men. J Androl 30(5):525–533. https://doi.org/10.2164/jandrol.108.006940
16. Greco E et al (2005) Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl 26(3):349–353
17. Sigman M et al (2006) Carnitine for the treatment of idiopathic asthenospermia: a randomized, double-blind, placebo-controlled trial. Fertil Steril 85(5):1409–1414
18. Rolf C et al (1999) Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. Hum Reprod 14(4):1028–1033
19. Kessopoulou E et al (1995) A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil Steril 64(4):825–831
20. Moilanen J, Hovatta O, Lindroth L (1993) Vitamin E levels in seminal plasma can be elevated by oral administration of vitamin E in infertile men. Int J Androl 16(2):165–166
21. Busetto GM et al (2018) Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: a double-blind placebo-controlled study. Andrologia 50(3):e12927. https://doi.org/10.1111/and.12927
22. Nadjarzadeh A et al (2011) Coenzyme Q 10 improves seminal oxidative defense but does not affect on semen parameters in idiopathic oligoasthenoteratozoospermia: a randomized double-blind, placebo controlled trial. J Endocrinol Invest 34(8):e224–e228. https://doi.org/10.3275/7572
23. Safarinejad MR (2011) Effect of pentoxifylline on semen parameters, reproductive hormones, and seminal plasma antioxidant capacity in men with idiopathic infertility: a randomized double-blind placebo-controlled study. Int Urol Nephrol 43(2):315–328. https://doi.org/10.1007/s11255-010-9826-4
24. Akmal M et al (2006) Improvement in human semen quality after oral supplementation of vitamin C. J Med Food 9(3):440–442
25. Lenzi A et al (2003) Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertil Steril 79(2):292–300
26. Wong WY et al (2002) Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. Fertil Steril 77(3):491–498
27. Omu AE, Dashti H, Al-Othman S (1998) Treatment of asthenozoospermia with zinc sulphate: andrological, immunological and obstetric outcome. Eur J Obstet Gynecol Reprod Biol 79(2):179–184