Antioxidants and Male Fertility

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Contributor: Pedro F. Oliveira

Spermatozoa are physiologically exposed to reactive oxygen species (ROS) that play a pivotal role on several sperm functions through activation of different intracellular mechanisms involved in physiological functions such as sperm capacitation associated-events. However, ROS overproduction depletes sperm antioxidant system, which leads to a condition of oxidative stress (OS). Subfertile and infertile men are known to present higher amount of ROS in the reproductive tract which causes sperm DNA damage and results in lower fertility and pregnancy rates. Thus, there is a growing number of couples seeking fertility treatment and assisted reproductive technologies (ART) due to OS-related problems in the male partner. Interestingly, although ART can be successfully used, it is also related with an increase in ROS production. This has led to a debate if antioxidants should be proposed as part of a fertility treatment in an attempt to decrease non-physiological elevated levels of ROS. However, the rationale behind oral antioxidants intake and positive effects on male reproduction outcome is only supported by few studies.

Keywords: assisted reproductive technologies ; sperm ROS ; pregnancy ; infertility ; antioxidants therapy ; reproductive outcome

1. Introduction

The mammalian spermatozoon is a cell with a high demand for energy to perform its function. Spermatozoa obtain their energy by two main metabolic pathways: glycolysis that occurs in the principal piece of the flagellum and oxidative phosphorylation (OXPHOS) that takes place on mitochondria located at the midpiece of the flagellum [1]. Spermatozoa contain between 50 and 75 mitochondria [2] and as with any other kind of cell that performs aerobic metabolism, is associated with the production of free radicals named reactive oxygen species (ROS) that include the hydroxyl radicals (•OH), superoxide anion ($•O_2^-$), hydrogen peroxide (H_2O_2), and nitric oxide (NO). These ROS are highly reactive molecules due to the presence of an unpaired electron in their outer shell. In addition, they have a very short half-life in the range of nanoseconds to milliseconds. ROS are produced as a consequence of natural cell machinery and participate in the normal function of a cell. However, when ROS production overcomes cellular antioxidant defenses surpassing a physiological range, they cause deleterious effects due to oxidative stress (OS) that results in oxidation of lipids, proteins, carbohydrates, and nucleotides [3].

Male subfertility and infertility have been associated with OS. Moreover, since infertile men have lower seminal plasma antioxidant capacity in comparison with fertile men, when higher levels of ROS occur, they led to an increase of lipid peroxidation (LPO) [4]. It is well described that when ROS overproduction occurs, it induces sperm DNA damage, although they have the potential to fertilize embryo development and fertility might be disturbed [5][6]. It is unclear how this is related with the fact that nowadays infertility is becoming a worldwide health problem, where one out of six couples are under fertility treatment and thus the use of assisted reproductive technologies (ART) to overcome this problem is growing exponentially. Nevertheless, ART is not harmless and is also associated with an increase of ROS production [7]. Although there is literature focused on the effects of consumption of oral substances with antioxidant properties on sperm parameters, the purpose of this review is to discuss the efficiency of antioxidant intake as a dietary supplement as well as an additive through ART procedures to counteract excessive ROS production that leads to infertility. We will also focus on the molecular mechanisms of action of those compounds with antioxidant activity in the male reproductive system, mainly reviewing literature that relates antioxidant treatment with ART, clinical pregnancy, and live birth as final outcomes.

2. Mechanism of ROS Defense in Spermatozoa

Spermatozoa differentiation is achieved during spermiogenesis as they gradually lose their cytoplasm. By the end of the process, the cytoplasm content is very small compared to other cells, where most of the space is occupied by DNA (sperm head). This special feature results in spermatozoa possessing low intracellular antioxidant activity consisting of superoxide dismutase (SOD), nuclear glutathione peroxidase (GPx), peroxiredoxin (PRDX), thioredoxin (TRX), and

thioredoxin reductase (TRD) [8]. Therefore, sperm ROS scavenger activity basically depends on the antioxidant content of the seminal plasma, which is formed mainly by a trio of enzymes where SOD converts superoxide anion (O_2^{-}.) to hydrogen peroxide (H_2O_2), preventing the formation of hydroxyl radical that is an inductor of LPO. However, the H_2O_2 generated is a strong membrane oxidant that is rapidly eliminated either by catalase (CAT) or GPx activities, giving H_2O as a product. Finally, seminal plasma also contains nonenzymatic antioxidant components such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), pyruvate, urate, taurine, and hypotaurine [9].

It should be noted that most ART involves washing steps, meaning that all the natural antioxidant defenses contained in seminal plasma are removed. Likewise, this also happens after natural insemination. During ejaculation, spermatozoa are surrounded by antioxidant molecules coming from seminal plasma but once the ejaculate reaches the vagina, seminal plasma is diluted, leading in both cases to spermatozoa facing ROS. Although spermatozoa possess antioxidant scavenger systems, it seems that they are not strong enough when ROS levels exceed physiological levels, subsequently making spermatozoa highly susceptible to OS.

3. Effects of Oral Antioxidant Intake on Male Reproductive Outcome

Currently, there is a growing trend of oral antioxidant intake to counteract high levels of ROS found in spermatozoa and seminal plasma of subfertile or infertile men. This hypothesis is supported by several works that describe an improvement of sperm parameters after oral antioxidant intake. Among those improvements, sperm concentration, motility, or decrease of DNA damaged are reported (Reviewed by [10]). However, only a few works have shown the effect of antioxidant therapy on fertility outcomes. Here, we discuss the major findings of oral antioxidant intake in reproduction outcome and its endpoints, such as fertility and live birth (summarized in **Table 1**).

Table 1. Effects of oral antioxidant intake on infertile men's reproductive outcome.

Antioxidant Type and Daily Dose	Period Intervention (months)	ART	Relevant Findings	Participants	Problem	Reference
Astaxantin (16 mg)	3	NI and IUI	↑ Pregnancy rate 54.5% (5/11) vs. 10.5% (2/19) placebo group	30	Infertile	[11]
LC (1 g twice) LAC (0.5 g twice)	3		↓ ROS levels ↑ Pregnancy (11.7%) in patients with abacterial-PVE with normal values of leucocytes It didn´t improve pregnancy (0%) in abacterial-PVE patients with high levels of leucocytes	54	PVE	[12]
Nonsteroidal anti-inflammatory + carnitine (Carnitene, 2 g + Nicetile 1 g) Carnitine (Carnitene, 2 g + Nicetile 1 g) Nonsteroidal anti-inflammatory Nonsteroidal anti-inflammatory + carnitine (Carnitene, 2 g + Nicetile 1 g)	2 + 2 4 4 4		23.1% pregnancy 0% pregnancy 6.2% pregnancy 3.8% pregnancy	98	PVE with ↑ levels of leucocytes	[13]
LC (3 g), LAC (3 g), LC (2 g) + LAC (1 g)	6	NI	↑ Total oxyradicals scavenging capacity of seminal fluid ↑ Sperm motility and concentration. Pregnancy rate was not modified	60	Asthenozoospermic	[14]

Antioxidant Type and Daily Dose	Period Intervention (months)	ART	Relevant Findings	Participants	Problem	Reference
LC (1 mg), fumarate (725 mg), LAC (500 mg), Fructose (1000 mg), CoQ10 (20 mg), Vitamin C (90 mg), Zinc (10 mg), Folic acid (200 µg), Vitamin B12 (1.5 µg)	6	NI	↑ Achieved pregnancy in treated men 22.2% (10/45) vs. 4.1% (2/49) non treated group	104	Oligo-and/or astheno-and/or teratozoospermia	[15]
LC fumarate (2 g), LAC (1 g) Clomiphene citrate (50 mg) and a complex of vitamins and microelements	3–4	NI	↑ Sperm concentration No modification in pregnancy rates	173	Oligo- and/or asteno- and/or teratozoospermia	[16]
LC fumarate (1 g), Acetyl-L- carnitine HCl (0.5 g) Fructose (1 g), Citric acid (50 mg), Vitamin C (90 mg), Zinc (10 mg), Folic acid (200 µg), Selenium (50 µg), Coenzyme Q-10 (20 mg) Vitamin B12 (1.5 µg)	6	NI	↑ Sperm concentration,% of sperm motile or progressive motility as well as sperm with normal morphology Treated men achieved 29% pregnancy versus 17.9% in the placebo group	90	After performed a varicocelectomy	[17]
Vitamin E (600 mg)	3	IVF	Improvement of zona pellucida binding test No effect on ROS levels No alteration on seminal plasma vitamin E levels	30	Infertile	[18]
Vitamin E (300 mg)	3	NI	21% of men had improved sperm motility and achieved pregnancy where 81.8% of pregnancies finished with a live birth	52	Asthenospermic	[19]
Vitamin E (200 mg)	1	IVF	↓ Sperm LPO ↑ Fertility rate: 19.3 ± 23.3 pre-treatment versus 29.1 ± 22.2 post-treatment	15	Normospermic infertile	[20]
Vitamin E (1 g) Vitmin D (1 g)	2	ICSI	76.3% respond to the treatment with ↓DNA damage ↑ Pregnancy rate (6.9 vs. 49.3%) ↑ Implantation rate (2.2 vs. 19.2%) Equal embryo quality	38	Infertile men non responding to ICSI	[21]
Vitamin E (400 IU) Selenium (200 µg)	3.5	NI	10.8% pregnancy	690	Infertile	[22]
Vitamin E (400 IU), Vitamin C (100 mg), Lycopene (6 mg), Zinc (25 mg), Selenium (26 µg), Folate (0.5 mg), Garlic (1000 mg)	3	IVF-ICSI	Doubled pregnancy rate (63.9 vs. 37.5%), Doubled implantation rate (46.2 vs. 24%) Doubled viable pregnancy rate (38.5 vs. 16%)	60	Infertile men with ↑ levels of DNA fragmentation and poor motility and membrane integrity	[23]

Antioxidant Type and Daily Dose	Period Intervention (months)	ART	Relevant Findings	Participants	Problem	Reference
Zinc sulphate (220 mg)	4	NI	21.4% (3/14) of patients achieved pregnancy Zinc levels were increased in seminal plasma	14	Human	[24]
Zinc sulphate (500 mg)	3	NI	Improved pregnancy (22.5%) vs. placebo (4.3%) Zinc levels were not modified on seminal plasma	100	Asthenozoospermic	[25]

NI: natural insemination, IVF: in vitro fertilization, ICSI: intracytoplasmic sperm injection, IU: international unit, PVE: prostate-vesiculo-epididymitis, LC: L-carnitine, LAC: L-acetyl-carnitine, LPO: lipid peroxidation, ↑ increase, ↓ decrease.

3.1. Carnitines

Carnitines are synthetized by the organism and found in seminal plasma at higher concentration than in spermatozoa. The l-carnitine (LC) isomer is the bioactive form [26] with a pivotal role in mitochondrial β-oxidation, acting as a shuttle of the activated long-chain fatty acids into the mitochondria [27] where l-acetyl-carnitine (LAC) is an acyl derivative of LC. Long-chain fatty acids provide energy to mature spermatozoa (with positive effects on sperm motility) and during maturation and the spermatogenic process [28]. Oral intake of LC (1 g twice/day) and LAC (0.5 g twice/day) for three months reduced ROS levels in spermatozoa and improved pregnancy (11.7%) in patients with abacterial prostate-vesiculo-epididymitis (PVE) with normal values of leucocytes, but it did not improve pregnancy at all (0%) in those PVE patients with high levels of leucocytes [12]. A year later, the same group tested patients diagnosed with abacterial PVE concomitant with high levels of leucocytes and showed that pretreatment for two months with a nonsteroidal anti-inflammatory followed by two months of carnitine oral intake achieved 23.1% pregnancy in comparison with the four-month carnitine intake group (0%), nonsteroidal anti-inflammatory group (6.2%), and the group receiving four-month nonsteroidal anti-inflammatory compounds and carnitines (3.8%) [13]. In another study, the effect of daily intake of LC (3 g), LAC (3 g), or a combination of LC (2 g) and LAC (1 g) was discriminated over six months and results were followed up 9 months after intervention in idiopathic asthenozoospermic men (n = 60) [14]. Treated men improved their total oxyradicals scavenging capacity of seminal fluid [14]. Overall, LAC or the combination of LAC + LC treatment had better improvement of sperm motility and concentration. Nevertheless, those patients with lower basal values of sperm motility had higher probability to respond to the treatment but pregnancy rate was not improved by any treatment in comparison with placebo control group [14]. Recently, coadministration of LC fumarate (2 g), LAC (1 g), and clomiphene citrate (50 mg) concurrently with vitamins and minerals in patients with idiopathic oligo- and/or asteno- and/or teratozoospermia (n = 173) enhanced sperm concentration specially in those patients with multiple impairment semen parameters (oligoasthenoteratozoospermic patients), but did not improve the morphology, progressive sperm motility neither pregnancy rates in comparison with control group [16]. A meta-analysis concerning carnitine used as an oral antioxidant therapy concluded that this molecule might be effective for improving pregnancy rates regarding the limits of patient inclusion criteria and the lower number of men evaluated in each study [29].

3.2. Vitamins

The interest of vitamin E and its use as antioxidant is due to its protective activity against ROS which subsequently decreases LPO, and therefore exerts positive effects on sperm functions, such as sperm concentration and motility [30]. However, its effects in fertility are less clear. For example, in a small clinical trial (n = 30), oral administration of vitamin E (300 mg twice daily) for three months raised the levels of vitamin E in blood serum, although human seminal plasma levels were not modified, questioning its possible effects on reproductive parameters [22]. Nevertheless, in this clinical trial, vitamin E treatment achieved an improvement of the zona pellucida binding test without any other improvement described, including ROS level [22]. Similarly, 15 normospermic infertile men after one month of daily consumption of 200 mg of vitamin E improved their fertilization rate (19.3 ± 23.3 pretreatment versus 29.1 ± 22.2 post-treatment) after IVF. Those results were associated with lower sperm LPO levels in comparison with preintervention values [24]. In another work, oral administration of vitamin E (100 mg thrice daily) to patients with asthenospermia (n = 52) established three different groups of men according to the results: (i) men without improvement of their sperm motility (40%); (ii) men with improved sperm motility but did not achieve pregnancy (39%); (iii) men with improved motility and achieved pregnancy (21%), of which 81.8% of pregnancies finished in live birth. The placebo control group did not achieve any pregnancies [19]. Later, daily intake of a combination of vitamin E and C (1 mg of each component) for two months in patients where

intracytoplasmic sperm injection (ICSI) had previously failed was studied (*n* = 38). The results showed two different populations: (i) those where the antioxidant treatment decreased the percentage of sperm DNA damage (*n* = 29) and (ii) those where the treatment did not affect this parameter (*n* = 9) [21]. The most interesting result was observed in the responsive group that after ICSI, the pregnancy rate (6.9 vs. 49.3%) and implantation rate (2.2 vs. 19.2%) were improved compared with the pretreatment group, although no differences were found in embryo quality [21]. In a nonplacebo-controlled and nondouble-blind design trial, daily intake of a combination of selenium (200 µg) and vitamin E (400 UI) followed for 3.5 months by infertile men (*n* = 690) achieved 10.8% spontaneous pregnancy [22].

Several studies have been performed looking for beneficial effects from a combination compounds with antioxidant activity. For example, a formulation using a mix of several compounds with antioxidant activity (vitamin C, vitamin E, carnitine, folic acid, lycopene, selenium, and zinc) was evaluated using a mouse Gpx5 knock-out (KO) subjected to a second stress: scrotal heat (KO + SH) (42 °C for 30 min) [30]. Although the exact ingestion quantity of this antioxidant combination could not be determined, their effects include the reversion of sperm DNA oxidation induced in KO + SH animals and protection of seminiferous tubules. The results showed that animals supplemented with KO + SH versus the nonsupplemented animals had double the fertilization rate (73.7 vs. 35.2%) and fetus reabsorption was halved (8.9 vs. 17.8%) [30]. In another trial, infertile human patients with oligo- and/ or astheno- and/or teratozoospermia with or without varicocele (*n* = 104) using a combination of antioxidants (vitamin C 90 mg, vitamin B12 1.5 µg, LC 1mg, fumarate 725 mg, LAC 500 mg, fructose 1000 mg, CoQ_{10} 20 mg, zinc 10 mg, and folic acid 200 µg) were studied for six months. The results showed that the individuals from the treated group, regardless of whether they suffered from varicocele or not, presented improved sperm concentration total sperm motility [15]. Moreover, after treatment, 22.2% (10/45) of supplemented patients achieved pregnancy, while in the control group, only 4.1% (2/49) of the couples were pregnant [15]. A close analysis of the men from the supplemented group revealed that only 4.8% (1/21) of patients suffering varicocele improved after treatment, while the nonvaricocele group achieved 37.5% (9/24) pregnancy [15]. A different group studied the effect of a commercial multiantioxidant supplement (vitamin E 400 IU, vitamin C 100 mg, lycopene 6 mg, zinc 25 mg, selenium 26 µg, folate 0.5 mg, garlic 1000 mg) for three months on 60 men with high levels of DNA fragmentation and poor sperm motility and membrane integrity [23]. The treatment achieved doubled pregnancy rate (63.9 vs. 37.5%), implantation rate (46.2 vs. 24%), and viable pregnancy rate (38.5 vs. 16%) versus the placebo group without any modification of any sperm parameters, fertilization, or embryo quality rates [23]. However, this work was later criticized because of the experimental design, particularly the low number of individuals in the trial, unequal distribution of individuals between the placebo (*n* = 16) and treatment groups (*n* = 36) and the suitability of the statistical analysis used [31].

Contradictory results were found when men were supplemented with different oral antioxidants after varicocelectomy. Oral intake of vitamin E (300 mg twice/day) for 12 months (*n* = 40) improved the sperm parameters of sperm concentration and the percentage of motile spermatozoa, although these data were not significant compared with control [32]. Recently, a multiple antioxidant combo was tested (l-carnitine fumarate 1 g, acetyl-l-carnitine HCl 0.5 g, fructose 1 g, citric acid 50 mg, vitamin C 90 mg, zinc 10 mg, folic acid 200 µg, selenium 50 µg, coenzyme Q-10 20 mg, and vitamin B12 1.5 µg) after varicocelectomy (*n* = 90) for six months [17]. Surgery improved the following sperm parameters: sperm concentration, percentage of motile spermatozoa or progressive motility, and spermatozoa with normal morphology. Moreover, treated men achieved 29% pregnancy versus 17.9% in the placebo group [17].

3.3. Zinc

Zinc is a metalloprotein cofactor for DNA transcription and protein synthesis. Moreover, zinc is necessary for the maintenance of spermatogenesis and optimal function of the testis, prostate, and epididymis [33], in addition to their antioxidant properties preventing LPO [34]. A trial using zinc sulphate as an antioxidant therapy administrated orally (250 mg twice daily) for three months reported an improvement in the reproductive outcome of asthenozoospermic men (*n* = 100), particularly in the sperm parameters of concentration, motility, and sperm membrane integrity (hypoosmotic swelling test). It was also noticed a decrease of antisperm antibodies on seminal plasma without modification of zinc levels on seminal plasma [25]. Pregnancies were also improved in couples where men underwent treatment when compared with placebo, 22.5% (11/49) versus 4.3% (2/48), respectively [25]. In another trial with only 14 patients and no control group, sperm parameters were improved after zinc treatment (220 mg daily for four months) and 21.4% (3/14) of patients achieved pregnancy and increase zinc levels on seminal plasma [24]. Although beneficial evidence has been found on reproductive outcome after zinc intake, the lower number of studies and subjects under treatment without a proper control does not allow further discussion of the possible positive effects of zinc intake on reproduction outcome.

3.4. Natural Compounds—Traditional Medicine

Natural compounds have been used traditionally to treat diseases. For instance, beneficial effects on reproductive outcome have been reported using products derived from tea (*Camelia sinensis* (L.)), which is the second most consumed

beverage after water [35]. For example, an in vitro experiment using green tea extract or epigallocatechin-3-gallate (EGCG) added to human spermatozoa media improved sperm capacitation hallmarks, such as tyrosine phosphorylation and cholesterol efflux, through the estrogen receptor pathway [36]. EGCG has been shown to have beneficial effects when extreme stresses are applied to male mice [37][38]. Interestingly, adverse effects induced by artificial testicular hyperthermia were ameliorated by oral administration of green tea extract [37]. Positive effects were visible after 28 days of heat stress induction, improving sperm concentration, percentage of motile and progressive spermatozoa, and sperm membrane integrity [37]. Another example of the beneficial effects of EGCG were described when intraperitoneal administration (50 mg/kg) protected against testicular injury induced by ionizing radiation in rats [38]. Thus, treated animals restored testicular function with an improvement in the number of pups by littler reducing LPO (TBARs) and protein carbonyl levels [38]. EGCG's mechanism of action is via the mitogen-activated protein kinase/BCL2 family/caspase 3 pathway [38]. In another work, the combination of two different tea extracts, white and green, where evaluated as additives to improve ART sperm of rats stored at room temperature. The authors found doubled levels of epigallocatechin (EGC) and EGCG in white tea in comparison with green tea [39], highlighting the variability associated with the type of tea extract used. Moreover, although both extracts had positive effects, the white tea extract had better ferric reducing antioxidant power than the green tea extract and the control. The beneficial effects were proportional to the concentration used, with 1 mg/mL of white tea extract being the best concentration tested for improving sperm survival and decreasing LPO over 72 hours of storage at room temperature [39]. Encouraged by the antioxidant effects on sperm parameters of white tea, the same group explored the oral administration potential of the extract to improve prediabetic type II (PreDM) male reproduction features known to be decreased due to oxidative stress [40]. PreDM is characterized by mild hyperglycemia, glucose intolerance, and insulin resistance and has been related with infertility or subfertility problems in males [41]. Consequently, using rat as an animal model, drinking white tea counteracted the negative effects of PreDM on the male reproductive tract. For example, white tea consumption improved testicular antioxidant power and decreased lipid peroxidation and protein oxidation [40]. Ingestion of white tea also restored sperm motility and restored sperm showing morpho-anomalies to normal levels [40].

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