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MINIREVIEW

Antioxidants and sperm DNA damage: a clinical perspective

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Abstract
Purpose Infertile men possess substantially more sperm DNA damage than do fertile men, damage that may impact negatively on reproductive outcomes. In this era of assisted reproductive technologies there is mounting concern regarding the safety of utilizing DNA-damaged spermatozoa in this setting. Therefore, it is important to identify strategies that may reduce sperm DNA damage. The purpose of this review is to discuss the rationale for antioxidant therapy in men with sperm DNA damage and to evaluate the data on the efficacy of dietary and in vitro antioxidant preparations on sperm DNA damage.
Methods We reviewed the literature on antioxidants and sperm DNA damage.
Results To date, the data suggest that dietary antioxidants may be beneficial in reducing sperm DNA damage, particularly, in men with high levels of DNA fragmentation. However, the mechanism of action of dietary antioxidants has not been established and most of the clinical studies are small. A beneficial effect of in vitro antioxidant supplements in protecting sperm DNA from exogenous oxidants has been demonstrated, however, the effect of these antioxidants in protecting sperm from endogenous ROS, gentle sperm processing and cryopreservation has not been established.

Keywords Sperm DNA fragmentation · Oxidative stress · Vitamins · Sperm washing · Male infertility

Capsule Dietary and in vitro antioxidant preparations may help reduce sperm DNA damage, however, larger, well-designed studies are needed to firmly establish the utility of antioxidants in this setting.

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Introduction

There is evidence to show that infertile men possess substantially more sperm DNA damage than do fertile men and that this DNA damage may adversely affect reproductive outcomes [1–4]. This is particularly relevant in an era where advanced forms of assisted reproductive technologies (ARTs) are commonly utilized (ARTs often bypass the barriers to natural selection), as there is some uncertainty and mounting concern regarding the safety of utilizing DNA-damaged spermatozoa in this setting [5]. Therefore, it is important to identify strategies that may reduce sperm DNA damage. The proposed strategies include eliminating testicular gonadotoxins or hyperthermia, treatment of semen or genital tract infections, correction of varicoceles and the use of antioxidants [6–8]. The purpose of this review is to explore the rationale for antioxidant therapy in men with sperm DNA damage and to present data on the efficacy of dietary and in vitro antioxidant preparations on sperm DNA damage.

Etiology of sperm DNA damage

The etiology of sperm DNA damage is multi-factorial and may be due to primary testicular or secondary (e.g. environmental) factors. Sperm DNA damage is believed to be the result of aberrant protamine expression, excessive

ROS (reactive oxygen species) generation and abortive apoptosis during spermatogenesis [9–12].

Relationship between ROS (reactive oxygen species) and sperm DNA damage

The association between sperm DNA damage and semen ROS is the basis for the use of antioxidants in the treatment of sperm DNA damage. High levels of ROS have been detected in the semen of 25% of infertile men [13, 14]. The levels of sperm-derived ROS (measured in sperm preparations having minimal leukocyte contamination) have been associated with sperm DNA damage, although no ROS threshold level above which sperm DNA damage is detected has been established [15–17]. Moreover, the levels of sperm DNA oxidation are higher in infertile compared to fertile men [18, 19]. Semen ROS are generated by spermatozoa (especially, defective or immature) and semen leukocytes [16, 20–23]. While the controlled release of low levels of ROS is necessary for normal sperm function, high levels of ROS can cause sperm dysfunction [23]. The susceptibility of human spermatozoa to oxidative stress stems primarily from the abundance of unsaturated fatty acids in the sperm plasma membrane. These unsaturated fatty acids provide fluidity that is necessary for membrane fusion events (e.g. the acrosome reaction and sperm-egg interaction) and for sperm motility. However, the unsaturated nature of these molecules predisposes them to free radical attack and ongoing lipid peroxidation throughout the sperm plasma membrane. Once this process has been initiated, accumulation of lipid peroxides occurs on the sperm surface and oxidative damage to DNA can ensue [6, 24, 25]. Studies have demonstrated that exogenous and endogenous ROS can induce sperm DNA damage in vitro, confirming that ROS may play a role in the etiology of sperm DNA damage in infertile men [25–27].

Semen antioxidants and sperm DNA damage

Seminal fluid is an important source of antioxidants (ROS scavengers) and is key in protecting spermatozoa from oxidative injury [14, 28, 29]. This is particularly important because spermatozoa have little cytoplasmic fluid (antioxidant enzymes are generally intracellular), virtually no capacity for protein synthesis and little antioxidant capacity [14]. The endogenous free radical scavenging enzymes in the male reproductive tract include superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) [14, 30–33]. These same antioxidant enzymes (SOD, catalase and GPX) are found in semen [34]. Moreover, several non-enzymatic antioxidants (e.g. vitamins C and E, hypotaurine, taurine, L-carnitine, lycopene) are also found in semen and this non-enzymatic component accounts for much of the total seminal antioxidant activity [14, 35].

Several clinical studies have evaluated the relationship between semen antioxidant levels and sperm DNA damage and have reported conflicting results. Some studies have shown that a deficiency in semen antioxidants is associated with sperm DNA damage, whereas, other studies have not observed such a relationship [6, 36–39]. Similarly, some studies have found that seminal antioxidant activity is reduced in infertile men with high levels of seminal ROS (relative to those with normal levels of ROS) whereas others have not shown this [14, 40–42]. To date, there are no studies to indicate a relationship between systemic antioxidant or vitamin deficiency and male infertility. Silver et al, 2005 evaluated a cohort of fertile men and did not identify any relationships between dietary antioxidant intake (vitamins C, E or ß-carotene) and sperm DNA damage [43]. Nonetheless, it is possible that a subset of infertile men may be at risk for antioxidant deficiency, particularly, vitamin C deficiency [44]. Moreover, infertile men with specific lifestyles may also be at risk for antioxidant or vitamin deficiency (e.g. smoking, increased alcohol intake, dieting)[45, 46].

Dietary antioxidant supplements and sperm DNA damage

In order to be active, a dietary antioxidant should be effectively absorbed and concentrated in reproductive tract organs. The antioxidant should also replete a deficiency (in the testis, epididymis or semen) and play a role in reproductive function. The antioxidant must either improve spermatogenesis and/or epididymal function, ultimately, resulting in improved sperm function and chromatin compaction and integrity. Alternatively, the antioxidant should enhance semen antioxidant capacity in order to reduce oxidative DNA damage.

There are a small number of reports on the effects of dietary antioxidant supplementation on sperm DNA integrity. In general, these are small studies that do not evaluate the mechanism of action of antioxidants: the only endpoint that is measured is the integrity of the sperm DNA or pregnancy rate. Moreover, most studies evaluate the effects of a short treatment course (with no long-term follow-up), are not randomized and fail to include a placebo-control group. Most of these clinical studies have evaluated men with high levels of sperm DNA damage. In these men, treatment with antioxidant supplements is generally associated with reduced levels of sperm DNA integrity and/or improved fertility potential (Table 1) [19, 47–52].

Table 1 Effect of dietary antioxidant supplements on sperm DNA integrity

Study	Patients/test	Treatment(s)	n	Results
Infertile men with high sperm DNA fragmentation levels				
Greco (47)	1 failed ICSI	vits C 1 g, E 1 g	38	Rx (2 months): ↓DD in 76%, 48% ICSI pregnancy
	TUNEL>15%			No control group.
Greco (48)	Infertility	vits C 1 g, E 1 g	32	Rx (2 months):↓ DD(22%→9%)
	TUNEL>15%		32	Placebo group: no effect on DD (22%→22%)
Menezo (49)	2 failed ICSI	vits C, E (400 mg)	57	Rx (90 days):↓ sperm %DFI (32→26%: by 19%)
	DFI>15%	Zinc, Se,		but ↑ sperm %HDS (17.5→25.5%: by 23%)
	Decond>15%	ß-carotene		No control group.
Tremellen (50)	Male Infert	Menevit (lycopene,vits C, E,	36	Rx (3 months): 39% ICSI pregnancy rate,
	TUNEL>25%	Zinc, Se, folate, garlic)		But no ↑ in embryo quality, no post-Rx DD
			16	Placebo group: 16% ICSI pregnancy rate
Gil Villa (51)	Pregn. loss	vits C, E	9	Rx (3 months): 6 (of 9) couples got pregnancy
	↑LPO or DFI	zinc, ß-carotene		No control group.
Infertile men (not selected on basis of DNA fragmentation)				
Piomboni (52)	Asthenosp.	vits C, E, ß-glucan	36	Rx (90 days): ↑motility & morph but not DD
	AO stain	papaya, lactoferrin	15	Control group: no effect
Kodama (19)	Male infert	Vit C, E (200 mg)	14	Rx (2 months):↓ in 8-OHdG ($1.5 \rightarrow 1.1/10^5$ dG)
	8-OHdG	glutathione (400 mg)	7	Control group: no change in 8-OHdG levels

8-OHdG 8-hydroxy-2-deoxyguanosine; *AO* acridine orange; *DD* DNA damage; *Decond* decondensation; *DFI* DNA fragmentation index; *LPO* lipid peroxidation; *Rx* Treatment; *Se* selenium; *TUNEL* terminal nucleotidyl transferase dUTP nick end labeling; *vit* vitamin

In 1991, Fraga et al. demonstrated that dietary vitamin C increases semen vitamin C levels and improves sperm DNA integrity (lowers DNA oxidation levels) in men with a vitamin C deficiency (on a vitamin C depleted diet). More recent studies of infertile men with high levels of sperm DNA damage (2 randomized controlled and 3 uncontrolled trials) have shown that antioxidant therapy is effective in improving sperm DNA integrity or pregnancy rates (Table 1). In men with unselected infertility, the effect of dietary antioxidants on sperm DNA integrity is equivocal with one of two controlled trials showing a benefit of antioxidants on sperm DNA integrity (Table 1).

In vitro antioxidants and sperm DNA damage

Several studies have examined the role of in vitro antioxidant supplementation in protecting the sperm DNA from oxidative damage. This is clinically relevant as sperm washing is routinely performed prior to ARTs (e.g. intrauterine insemination and in vitro fertilization) and the process may result in injury to the sperm DNA, particularly, as spermatozoa are now vulnerable to oxidative stress because seminal plasma (rich in antioxidants) has been removed in the process [53]. However, it is important to note that subpopulations of spermatozoa will exhibit variable susceptibility to oxidative stress: the

Table 2 Role of in vitro antioxidant supplements in protecting sperm DNA from exogenous ROS

Study	Assay	Exogenous ROS	Antioxidant supplement and results
Lopes (55)	TUNEL	X+XO	GSH+hypotaurine protect spz from X+XO-induced DD
			Catalase and n-acetylcysteine individually protect spz from X+XO induced DD
Potts (56)	TUNEL	H_2O_2+Fe+ADP	S. plasma (>60%v/v) lowers oxidative spz damage (↓DD, LPO)
Sierens (57)	COMET	H_2O_2	Isoflavones, vit C & E protect spz from H_2O_2-induced DD
			(Isoflavones: genistein, equol). Dose effect noted.
Russo (58)	COMET	(1) H_2O_2,	Propolis lowers oxidative spz damage (↓LPO, DD, LDH)
		(2) Benzopyrene,	(Propolis – a natural resinous hive product)
		(3) H_2O_2+Fe+ADP	

ADP adenosine diphosphate; *COMET* single cell gel electrophoresis; *DD* DNA damage; *DFI* DNA fragmentation index; Fe iron; *GSH* glutathione; *LDH* lactate dehydrogenase; *LPO* lipid peroxidation; *S.* plasma seminal plasma; *Spz* sperm; *TUNEL* terminal nucleotidyl transferase dUTP nick end labeling; *X* xanthine; *XO* xanthine oxidase

Table 3 Role of in vitro antioxidant supplements in protecting sperm DNA from stimulated endogenous reactive oxygen species (ROS) generation

Study	Assay	ROS stimulant	Antioxidant supplement and results
Twigg (25)	IS NTL	NADPH	Vit E, SOD, catalase, hypotaurine, albumin all ineffective in protecting spz DNA from endogenous ROS
Cemeli (59)	COMET	Estrogens (1 hr 37C)	Flavonoid (Kaempferol) protects sperm from estrogen-induced oxidative DD. (Flavonoids: only)
Dobrzynska (60)	COMET	DES, T3,T4, NA (1 hr 37C)	Flavonoids & catalase protect spz from stimulant-induced oxidative DD. (Flavonoids: Kaempferol, Quercetin).
Anderson (61)	COMET	Estrogens	Catalase protects spz from estrogen-induced oxidative DD SOD and vit C less effective. (Estrogens: equol, daidzein, genistein, DES, E2)

COMET alkaline single cell gel electrophoresis; *DD* DNA damage; *ISNTL* in situ nick translation assay; *LPO* lipid peroxidation; *NA* noraqdrenaline; *ROS* reactive oxygen species; *SOD* superoxide dismutase; *Spz* sperm; *T3* triiodothyronine; *T4* thyroxine; *vit* vitamin

DNA of normal spermatozoa is reportedly less susceptible to gentle processing techniques than is the DNA of abnormal or immature spermatozoa [21, 54].

The studies on in vitro antioxidant supplementation have looked at the role of antioxidants in protecting sperm from exogenous and endogenous ROS, and, from the effects of semen processing and cryopreservation. Antioxidants (e.g. vitamins C and E, catalase, glutathione) have been shown quite clearly to protect sperm DNA from the effects of exogenous ROS (see Table 2) [55–58]. This is of clinical relevance as many of the semen samples contain leukocytes and these cells have the potential to generate exogenous ROS. In contrast, antioxidants appear to be of limited value in protecting the DNA of normal spermatozoa from endogenous ROS production (e.g. NADPH-induced or centrifugation-induced) (see Table 3) [25, 59–61]. In samples with poor morphology and poor sperm chromatin compaction, antioxidants may protect the sperm DNA from endogenous ROS production, as these samples are more vulnerable to oxidative stress [21, 54]. In general, antioxidants appear to be of limited value in protecting sperm DNA from gentle semen processing (e.g. incubation or density-gradient centrifugation) (see Table 4) [62–65]. In some cases, antioxidants supplementation in vitro (e.g. combination of vitamins C and E) may cause sperm DNA damage [63, 64]. The one study evaluating the effects of sperm cryopreservation suggests that antioxidants (vitamin E) do not protect sperm DNA in this setting [66].

In summary, the data suggest that ROS appear to play an important role in the generation of sperm DNA damage. Although in vitro studies have demonstrated a beneficial effect of antioxidant supplements in protecting sperm DNA from exogenous oxidants, the effect of these antioxidants in protecting sperm from endogenous ROS, gentle sperm processing and cryopreservation has not been established. The data suggest that dietary antioxidants may be beneficial in reducing sperm DNA damage, particularly, in men with high levels of DNA fragmentation. However, the mechanism of action of dietary antioxidants has not been established and most of the clinical studies are small.

Table 4 Role of in vitro antioxidant supplements in protecting sperm DNA from semen processing

Study	Assay	Semen processing	Antioxidant supplement and results
Chi (62)	COMET	Centrifugation (1000 rpm *x*2) + 1 hr incubation	EDTA or catalase lower centrifugation-induced sperm ROS EDTA or catalase lower centrifugation-induced sperm DD EDTA or datalase have no protective effect on LPO
Donnelly (63)	COMET	Percoll DGC	Vit C or E do not lower baseline sperm ROS & DD
Hughes (64)	COMET	Percoll DGC	Vitamins C, E or urate lower sperm DD after DGC Vitamins C+E or AC increase sperm DD after DGC
Donnelly (65)	COMET	Percoll DGC ± H_2O_2	GSH, hypotaurine or both do not alter baseline sperm DD

AC Acetyl cysteine; *COMET* alkaline single cell gel electrophoresis; *DD* DNA damage; *DGC* density gradient centrifugation; *GSH* glutathione; *LPO* lipid peroxidation; *ROS* reactive oxygen species; *vit* vitamin

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