Oxidative Stress and Male Infertility 124

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Abstract

Infertile men have higher levels of semen reactive oxygen species (ROS) than do fertile men. High levels of semen ROS can cause sperm dysfunction, sperm DNA damage, and reduced male reproductive potential. These observations suggest that oxidative stress (OS) is an important cause of male infertility. This has led clinicians to treat infertile men with the aim of reducing seminal OS (e.g., with antioxidant supplements). The purpose of this review is to discuss the role of ROS in male infertility and the rationale for antioxidant therapy in these men. To date, most clinical studies suggest that dietary and in vitro antioxidant supplements are beneficial in terms of improving sperm function. However, the exact mechanism of action of dietary antioxidants and the optimal dietary supplement has not yet been established.

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Introduction

Reactive oxygen species (ROS) are ubiquitous in aerobic biologic systems and are formed as a byproduct of oxygen metabolism. Small amounts of semen ROS are necessary for the initiation of critical sperm functions, including capacitation and the acrosome reaction (de Lamirande and Gagnon 1993; Griveau et al. 1994). High ROS levels produce a state known as oxidative stress that can lead to biochemical or physiologic abnormalities with subsequent cell dysfunction or cell death (Aitken and Fisher 1994). Seminal oxidative stress (OS) is believed to be one of the main factors in the pathogenesis of sperm dysfunction and sperm DNA damage in male infertility (Chen et al. 2012; Aitken et al. 2010; Aitken and Roman 2008; Iwasaki and Gagnon 1992). Several intrinsic (testicular) and extrinsic factors (external stressors) can promote reactive oxygen species (ROS) generation in the testis and in the post-testicular (e.g., epididymal) environment, resulting in defective spermatogenesis and sperm dysfunction (see Fig. 124.1) (Aitken and Roman 2008). Indeed, it is estimated that 25 % of infertile men possess high levels of semen ROS, whereas fertile men do not have high levels of semen ROS (Chen et al. 2012; Iwasaki and Gagnon 1992; Zini and Sigman 2009; Agarwal et al. 2004). The detrimental effects of reactive oxygen species (ROS) on spermatozoa were suggested more than 60 years ago with the demonstration that exposing sperm to oxygen results in sperm toxicity (MacLeod 1943). Later studies confirmed that human spermatozoa and semen leukocytes have the capacity to generate ROS (Iwasaki and Gagnon 1992; Alvarez et al. 1987; Aitken et al. 1989; Zini et al. 1993).

ROS such as the superoxide anion ($O_2^{\bullet -}$) are generated as a byproduct of aerobic metabolism (Grisham and McCord 1986). The superoxide anion can then either spontaneously dismutate or be converted to H_2O_2 by the enzyme superoxide dismutase (SOD). The enzymes catalase or glutathione peroxidases will then convert H_2O_2 to water and oxygen, eradicating the ROS. Recently, investigators in our laboratory have detected active peroxiredoxins (a new family of antioxidant enzymes) in human semen and have shown that the low levels of these antioxidants in spermatozoa of infertile men is associated with impaired sperm motility, lipid peroxidation, and sperm DNA damage (O'Flaherty and de Souza 2011; Gong et al. 2012). These studies have also shown that unlike what is observed in fertile men, the peroxiredoxins in spermatozoa of infertile men are mostly oxidized, suggesting that the redox state of peroxiredoxins is important in the protection of human spermatozoa against oxidative stress (Alvarez and Storey 1983). Alternatively, both superoxide anion and hydrogen peroxide can be neutralized by nonenzymatic antioxidants such as albumin, glutathione, hypotaurine, taurine, as well as vitamins C and E (Alvarez and Storey 1983).

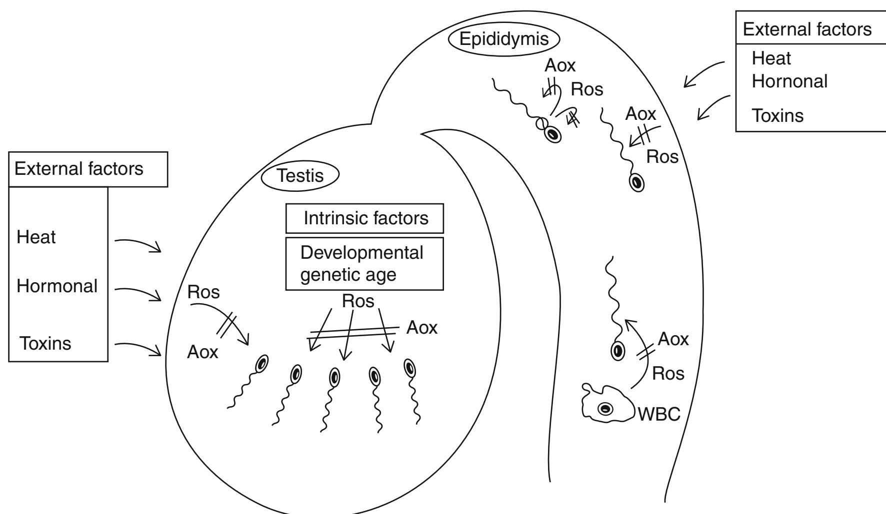

Figure 124.1 Several intrinsic (developmental, genetics, age) and extrinsic factors (e.g., heat, hormonal imbalance, toxins) can promote reactive oxygen species (*ROS*) generation in the testis and in the post-testicular (e.g., epididymal) environment. In the testis, ROS can interfere with normal spermatogenesis resulting in the generation of abnormal or immature sperm. In the post-testicular environment, ROS produced from various sources (epithelium, leukocytes, immature spermatozoa) can cause defective sperm function and DNA damage. Antioxidants may act at several sites to neutralize some of these ROS-mediated effects. *AOX* antioxidants, *ROS* reactive oxygen species, *WBC* white blood cell

Seminal OS results from an imbalance between ROS production and ROS scavenging by seminal antioxidants. Although a controlled production of these ROS is required for sperm physiology (sperm hyperactivation, capacitation, and acrosome reaction) and for natural fertilization (Aitken et al. 1995a; Griveau and Le Lannou 1997; de Lamirande et al. 1997), the excessive production of ROS by immature germ cells and leukocytes causes sperm dysfunction (lipid peroxidation, loss of motility, and sperm DNA damage) (Aitken and Clarkson 1987; de Lamirande and Gagnon 1992a; de Lamirande and Gagnon 1992b). Spermatozoa are particularly susceptible to oxidative injury due to the abundance of plasma membrane polyunsaturated fatty acids (de Lamirande et al. 1997; Aitken and Clarkson 1987; de Lamirande and Gagnon 1992a; de Lamirande and Gagnon 1992b; Zini et al. 2000). 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 lipid peroxidation has been initiated, accumulation of lipid peroxides occurs on the sperm surface with ensuing sperm dysfunction (loss of sperm motility, sperm DNA damage) and sperm death (Alvarez et al. 1987; Aitken et al. 1989; Twigg et al. 1998; Aitken et al. 1998 new Aitken).

Recently, investigators have shown that the products of lipid peroxidation can themselves stimulate free radical generation by the sperm mitochondria, thus creating an ongoing cycle of ROS production and cellular damage (Aitken et al. 2012).

Seminal Antioxidant Capacity and Sperm Dysfunction

Both seminal plasma and spermatozoa contain antioxidants designed to protect spermatozoa from OS, especially, at the post-testicular level (Agarwal et al. 2004; Tremellen 2008; Saleh and Agarwal 2002). Seminal plasma is a rich source of high molecular weight enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidases, and peroxiredoxins), and a deficiency in these enzymes has been reported to cause sperm DNA damage and male infertility (O'Flaherty and de Souza 2011; Gong et al. 2012; de Lamirande et al. 1997; Chabory et al. 2009; Weir and Robaire 2007; Jelezarsky et al. 2008). Seminal fluid also contains nonenzymatic antioxidants (ascorbic acid, α-tocopherol, pyruvate, glutathione, L-carnitine, taurine, hypotaurine) that constitute the bulk of seminal antioxidant capacity (Zini et al. 1993; Smith et al. 1996; Kobayashi et al. 1991; Zini and Schlegel 1996). In addition, urate, pyruvate, albumin, beta-carotenes, and ubiquinol have been detected in seminal plasma, and these are believed to protect spermatozoa from oxidative injury (Aitken and Fisher 1994; Thiele et al. 1995; Kalthur et al. 2008; Bilodeau et al. 2002; Tauber et al. 1975).

A number of investigators have shown that seminal antioxidant capacity is suppressed in infertile men with high ROS levels compared to men with normal levels of ROS (Smith et al. 1996; Lewis et al. 1997; Sanocka et al. 1996). However, it is unclear whether reduced semen antioxidant capacity necessarily causes sperm dysfunction (including sperm DNA damage) (Aitken and Roman 2008; Appasamy et al. 2007; Verit et al. 2006). Indeed, there is some controversy as to whether the high ROS levels detected in the semen of infertile men are due to increased ROS production, decreased ROS scavenging capacity, or both (Zini et al. 1993; Lewis et al. 1995). If the high semen ROS levels are due (at least in part) to a decreased ROS scavenging capacity of semen, it would support the use of dietary antioxidant supplementation (Zini et al. 1993; Lewis et al. 1995).

Although a relationship between male infertility and systemic antioxidant deficiency has not been reported to date, it is possible that a subset of infertile men may be at risk for antioxidant deficiency, particularly vitamin C deficiency (Hampl et al. 2004). We suspect that infertile men with specific lifestyles (e.g., smoking, increased alcohol intake, dieting) may be at high risk for antioxidant or vitamin deficiency, but this remains to be tested (Jacob 1990; Ryle and Thomson 1984). Investigators have evaluated dietary antioxidant intake

(vitamins C and E or ß-carotene) and sperm DNA damage in a cohort of fertile men but failed to identify any relationships between these parameters (Silver et al. 2005).

Detection of Seminal ROS

Semen ROS are generally measured by detection of chemiluminescence. Briefly, this is done by incubating fresh semen or sperm suspensions with a redox-sensitive, light-emitting probe (e.g., luminol) and by measuring the light emission over time with a light meter (luminometer). Sperm-specific (e.g., PMA) or leukocyte-specific (e.g., FMLP) stimulants can enhance the chemiluminescent signal (Krausz et al. 1994). Sperm ROS can also be measured by using cellular probes coupled with flow cytometry (Marchetti et al. 2002).

Several independent researchers have assessed the clinical value of semen ROS determination. Aitken et al. observed an inverse relationship between semen ROS levels and spontaneous pregnancy outcome in a cohort of 139 untreated infertile couples (Aitken et al. 1991). However, no other studies have supported these findings.

Semen ROS determination possesses some inherent weaknesses as a clinical test. Firstly, there is no established cutoff or threshold semen ROS level or value that can be used to predict reproductive outcomes. Secondly, semen ROS determination requires the use of fresh semen samples, making it impossible to send samples to a distant reference laboratory for ROS determination or assessment of interlaboratory variability. Finally, the protocol for ROS determination varies from laboratory to laboratory.

Several studies have evaluated the relationship between semen ROS and fertilization at IVF (Krausz et al. 1994; Marchetti et al. 2002; Yeung et al. 1996; Sukcharoen et al. 1996; Moilanen et al. 1998; Zorn et al. 2003; Saleh et al. 2003; Hammadeh et al. 2006). The value of semen ROS determination in the context of IVF is inconclusive (only half of the studies support its application). Some of these studies have reported a significant inverse relationship between sperm ROS levels and fertilization rate at conventional IVF. However, an equal number of studies have failed to demonstrate a significant relationship between ROS levels and fertilization rates (Table 124.1). Therefore, the clinical value of semen ROS determination in predicting IVF outcome remains unproven.

The available studies fail to demonstrate that semen ROS determination is of any significant clinical value. Semen ROS levels may be useful in predicting spontaneous pregnancy outcome, but there is only one supporting study in this respect. Although additional studies would help define the clinical utility of semen ROS testing, it is unlikely that such studies will be conducted because the test is labor intensive and requires testing fresh semen. Other surrogate tests of semen oxidative stress (e.g., 8-hydroxy-2-deoxyguanosine – a measure of oxidative DNA damage) may be better candidate tests of semen OS as they can be automated (e.g., flow cytometry) and can be applied to cryopreserved samples.

Table 124.1 Relationship between ROS levels in washed sperm samples and fertilization at IVF

Study	*n*	*r*	TV%	Method	Enhancer	Stimulant
Krausz et al. 1994	27	NS	–	Chemiluminescence	Luminol	FMLP + PMA
Yeung et al. 1996	75	NS	–	Chemiluminescence	Luminol	–
Sukcharoen et al. 1996	73	−0.22	–	Chemiluminescence	Luminol	–
Sukcharoen et al. 1996	73	−0.30	–	Chemiluminescence	Luminol	FMLP
Sukcharoen et al. 1996	73	−0.28	–	Chemiluminescence	Luminol	PMA
Moilanen et al. 1998	86	NS	–	Chemiluminescence	Luminol	FMLP + PMA
Marchetti et al. 2002	45	NS	–	Flow cytometry	–	–
Zorn et al. 2003	41	Inverse	–	Chemiluminescence	Luminol	–
Saleh et al. 2003	10	−0.59	–	Chemiluminescence	Luminol	–
Hammadeh et al. 2006	26	−0.26	–	Colorimetric assay	–	–

PMA 12-myristate, 13-acetate phorbol ester, *FMLP N*-formyl-methionyl-leucyl-phenylalanine, *n* number of treatment cycles, *r* Spearman rank order or Pearson correlation between ROS levels and fertilization, *TV* threshold value of ROS levels, *NS* not significant ($P > 0.05$)

Treatment of Oxidative Stress

Treatment of oxidative stress should first involve strategies to reduce or eliminate stress-provoking conditions including smoking, varicocele, genital infection, gonadotoxins, and hyperthermia. The rationale for treating infertile men with oral antioxidants is based on the premise that seminal oxidative stress (common in infertile men) is due in part to a deficiency in seminal antioxidants. The practice of prescribing oral antioxidant is supported by the lack of serious side effects related to antioxidant therapy, although few studies have carefully evaluated the risk of overtreatment with antioxidants (Henkel 2011). Ideally, an oral antioxidant should reach high concentrations in the reproductive tract and replete a deficiency of vital elements important for spermatogenesis. Additionally, the antioxidant supplement should augment the scavenging capacity of seminal plasma and reduce the levels of semen ROS (Chen et al. 2012). However, the levels of semen ROS should not be entirely suppressed (by oral antioxidants) as this may impair normal sperm functions (e.g., sperm capacitation and hyperactivation) that normally require low levels of ROS (Aitken et al. 1995a; de Lamirande et al. 1997).

To date, over 100 clinical and experimental studies have examined the effect of antioxidants on sperm parameters. Despite this large body of literature, it is not possible to establish firm conclusions regarding the optimal antioxidant treatment for infertile men because the published studies report on different types and doses of antioxidants, the studies are small, the end points vary, and few of the studies are placebo controlled (Agarwal et al. 2004; Tremellen 2008). Moreover, the presumed mechanism of action of antioxidants in the treatment of male infertility (i.e., suppression of seminal OS) has not been confirmed because few studies have evaluated seminal OS and/or antioxidant capacity before and after treatment (Comhaire et al. 2005).

Effect of Oral (Dietary) Antioxidants on Sperm Dysfunction and DNA Damage

While there is a good body of literature on the effect of oral antioxidants on sperm parameters (including sperm DNA integrity), no study has established the optimal dose, duration of treatment, or subpopulation of infertile patients who might benefit most from antioxidant therapy (isolated asthenozoospermia, oligoasthenoterato-zoospermia, sperm DNA damage, or all) (Lombardo et al. 2011). Many small, uncontrolled studies have shown a significant improvement in semen parameters following different doses and types of antioxidant therapy (Agarwal et al. 2004; Tremellen 2008). The most commonly studied oral antioxidants (or antioxidant enzyme cofactors) include vitamin C, vitamin E, selenium, zinc, glutathione, L-carnitine, and N-acetylcysteine.

The randomized controlled trials (RCTs) on antioxidant therapy for male infertility generally demonstrate that treatment with antioxidants has a beneficial effect (in terms of semen parameter improvements), whereas no significant effect is seen in the placebo group (see Tables 124.2 and 124.3) (Comhaire et al. 2005; Suleiman et al. 1996; Lenzi et al. 1993; Keskes-Ammar et al. 2003; Balercia et al. 2005; Scott et al. 1998; Cavallini et al. 2004; Ciftci et al. 2009; Dawson et al. 1992; Ebisch et al. 2006; Hawkes et al. 2009; Lenzi et al. 2003; Mahajan et al. 1982; Omu et al. 2008; Omu et al. 1998; Piomboni et al. 2008a; Galatioto et al. 2008; Safarinejad and Safarinejad 2009; Wong et al. 2002; Kessopoulou et al. 1995; Moilanen et al. 1993; Rolf et al. 1999; Greco et al. 2005a; Sigman et al. 2006; Paradiso Galatioto et al. 2008). The variable treatment outcomes in the different studies could be due to differences in antioxidant supplements (e.g., vitamins C and E, selenium, zinc, L-carnitine), dosages, duration of treatment, and patient population (Agarwal et al. 2004; Tremellen 2008). This topic has recently been evaluated in a systematic fashion and published in a Cochrane Review (Showell et al. 2011). The results of the systematic review indicate that oral antioxidant supplements (for male infertility) can help increase pregnancy rates.

One randomized controlled trial (RCT) evaluated the effects of vitamin C alone and reported a significant improvement in sperm parameters in the treatment arm only (Dawson et al. 1992). Six RCTs evaluated the effects of vitamin E alone or in combination with vitamin C or selenium. Two of these studies reported a significant improvement in sperm motility (Suleiman et al. 1996; Keskes-Ammar et al. 2003), and one reported a significant improvement in sperm DNA integrity (Greco et al. 2005a) in the treatment arm only. In contrast, three RCTs reported no significant improvement in sperm parameters after vitamin E $\pm$ C treatment (Kessopoulou et al. 1995; Moilanen et al. 1993; Rolf et al. 1999) although sperm-zona binding improved in one of these studies (Kessopoulou et al. 1995). Five RCTs evaluated the effects of zinc alone or in combination with folic acid, and all five reported a significant improvement in sperm parameters in the treatment arm only (Ebisch et al. 2006; Mahajan et al. 1982; Omu et al. 2008; Omu et al. 1998; Wong et al. 2002). Three RCTs evaluated the effects of selenium alone or in combination with N-acetylcysteine, and two of the three studies reported a significant improvement in

Table 124.2 Summary of studies (RCT) with positive effect of oral antioxidants on sperm parameters

Study	Antioxidant and dose	Duration of treatment	Study population	Sample size	Improvement
Mahajan et al. 1982	Zinc 50 mg	6 months	Gonadal dysfunction in uremic patients	n-treated 10 n-control 10	Concentration
Dawson et al. 1992	Vitamin C, 1 g/d or 200 mg/d	1 month	Heavy smokers	n-treated 50 n-control 25	Sperm quality Sperm parameters
Lenzi et al. 1993	Glutathione 600 mg alternate days	2 months	Infertility with varicocele or genital tract infection	n-treated 10 n-control 10 crossover	Motility, morphology
Suleiman et al. 1996	Vitamin E, 300 mg	6 months	Asthenospermia	n-treated 52 n-control 35	MDA, motility
Scott et al. 1998	Selenium 100 Mg or/with 1 mg vit A, 10 mg vit C, 15 mg vit E	3 months	OAT, subfertile	n-treated 46 n-control 18	Motility
Omu et al. 1998	Zinc 500 mg	3 months	Asthenospermia	n-treated 49 n-control 48	Concentration Motility
Wong et al. 2002	Folic acid 5 mg Zinc 66 mg	26 weeks	Subfertile men	n-treated 94 n-control 99	Concentration
Keskes-Ammar et al. 2003	Vitamin E 400 mg, selenium 225 Mg	3 months	Infertility	n-treated 28 n-control 20	MDA, motility, concentration
Lenzi et al. 2003	L-Carnitine, 2 gm.	6 months	OAT	n-treated 43 n-control 43 crossover	Concentration, motility
Cavallini et al. 2004	L-Carnitine 2 g/d +/− Acetyl-L-carnitine 1 g/d +/− cinnoxicam 1x30mg	6 months	Idiopathic OAT Varicocele-associated OAT	n-treated 118 n-control 207	Concentration Motility Morphology (except in high-grade varicocele)
Balercia et al. 2005	LC 3 g/d, LAC 3 g/d, a combination of LC 2 g/d and LAC 1 g/d,	6 month	Asthenospermia	n-treated 44 n-control 15	Motility
Comhaire et al. 2005	Astaxanthin 16 mg	3 months	Unexplained infertility	n-treated 11 n-control 19	Motility Concentration
Ebisch et al. 2006	Folic acid 5 mg Zinc 66 mg	26 weeks	Subfertile	n-treated 47 n-control 40	Concentration
Omu et al. 2008	Zinc 400 mg +/− vitamins E 20 mg and C 5 mg	3 months	Asthenospermia	n-treated 37 n-control 8	Mainly motility Concentration, morphology

(*continued*)

Table 124.2 (continued)

Study	Antioxidant and dose	Duration of treatment	Study population	Sample size	Improvement
Piomboni et al. 2008	Beta-glucan 20 mg, papaya 50 mg, lactoferrin 97 mg, and vitamin C 30 mg and vitamin E 5 mg	3 months	Asthenoteratozoo-spermia	n-treated 36	Motility
				n-control 15	Morphology
Galatioto et al. 2008	NAC600mg + vitamins minerals		Persistent oligospermia	n-treated 20	Concentration
				n-control 22	
Safarinejad & Safarinejad 2009	Selenium 200 Mg +/− N-acetylcysteine 600 mg	26 weeks	Asthenospermia	n-treated 468	Motility
				n-control 118	Concentration
					Morphology
Ciftci et al. 2009	NAC 600 mg	3 months	Idiopathic infertility	n-treated 60	Motility
				n-control 60	Viscosity
					Volume

MDA malondialdehyde, *OAT* oligoasthenoteratospermia, *LC* L-carnitine, *LAC* L-acetylcarnitine, *NAC* N-acetylcysteine

Table 124.3 Summary of studies (RCT) with no effect of oral antioxidants on sperm parameters

Study	Antioxidant and dose	Duration of treatment	Study population		No improvement
Kessopoulou et al. 1995	Vitamin E, 600 mg	3 months	Infertility with high ROS	Crossover Treated and controls(30)	Concentration, motility, morphology
Moilanen et al. 1993	Vitamin E 100 mg	3 months	Unexplained infertility – IUI	n-treated 6	Concentration
				n-control 9	Motility
					Morphology
Rolf et al. 1999	Vitamin C 1000 mg, vitamin E 800 mg	56 days	Asthenospermia	n-treated 15	Concentration, motility, morphology, viability
				n-control 16	
Greco et al. 2005a	Vitamins C and E, 1 g/d	2 months	Idiopathic infertility	n-treated 32	Concentration
				n-control 32	Motility
					Morphology
Sigman et al. 2006	Carnitine 1000 mg, L-acetylcarnitine 500 mg	24 weeks	Asthenospermia	n-treated 12	Motility
				n-control 9	
Hawkes et al. 2009	Selenium 300 Mg/d	48 weeks	Normozoospermia	n-treated 20	Motility
				n-control 22	Morphology

ROS reactive oxygen species

sperm parameters in the treatment arm only (Scott et al. 1998; Hawkes et al. 2009; Safarinejad and Safarinejad 2009). Four RCTs evaluated the effects of L-carnitine alone or in combination with L-acetylcarnitine, and three of the four reported a significant improvement in sperm parameters in the treatment arm only (Balercia et al. 2005; Cavallini et al. 2004; Lenzi et al. 2003; Sigman et al. 2006). Three RCTs evaluated the effects of N-acetylcysteine alone or in combination with selenium, and all three reported a significant improvement in sperm parameters in the treatment arm only (Ciftci et al. 2009; Safarinejad and Safarinejad 2009; Paradiso Galatioto et al. 2008).

Several investigators have examined the effect of antioxidant therapy on sperm DNA integrity because sperm DNA damage may be caused, at least in part, by oxidative stress (Tremellen 2008; Zini and Schlegel 1996; Appasamy et al. 2007; Piomboni et al. 2008a; Gil-Villa et al. 2009; Greco et al. 2005b; Greco et al. 2005c; Kodama et al. 1997; Menezo et al. 2007; Tremellen et al. 2007; Tunc et al. 2009; Piomboni et al. 2008b). However, it is not possible to determine if sperm damage is due to increase seminal ROS production or deficient seminal antioxidant capacity unless one measures both ROS and total antioxidant capacity – TAC (Mahfouz et al. 2010). Nonetheless, sperm DNA damage is a more reliable outcome measure than sperm concentration or motility because measures of sperm DNA damage exhibit a lower degree of biological variability than conventional semen parameters (Zini et al. 2001; Evenson et al. 1991). Moreover, it is easier to implement testing of sperm DNA damage because the test can be applied to cryopreserved samples. Treatment with oral antioxidants has generally been associated with improvement in sperm DNA integrity and in some cases pregnancy rates after assisted reproduction, although most of these studies are small and few are randomized placebo-controlled trials (see Table 124.4) (Chen et al. 2012). To date, none of the studies on sperm DNA damage and oral antioxidants have estimated seminal oxidative stress, seminal vitamin levels, or used oxidative DNA damage (e.g., by estimation of 8-hydroxy-2′-deoxyguanosine [8OHdG]) as a selection criterion for monitoring the response to antioxidant treatment (Chen et al. 2012; Aitken et al. 2010; De Iuliis et al. 2007). As such, the precise mechanism of action of these antioxidant supplements on sperm DNA quality is unknown.

Effect of In Vitro ROS on Sperm Dysfunction and DNA Damage

The addition of ROS to sperm preparations (exogenous ROS) or the activation of intrinsic sperm ROS production (endogenous ROS) in vitro has been associated with the development of lipid peroxidation, sperm dysfunction, and sperm DNA damage (Alvarez et al. 1987; Aitken et al. 1989; Twigg et al. 1998; Aitken et al. 1998; Aitken and Baker 1995; Aitken et al. 1995b; Anderson et al. 2003). This is particularly important in the context of in vitro fertilization where 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

Table 124.4 Effect of dietary antioxidant supplements on sperm DNA integrity

Study	Patients/test	Treatment(s)	n	Results
Infertile men with high sperm DNA fragmentation levels or oxidative stress				
Greco et al. 2005b	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 et al. 2005a	Infertility	Vits C 1 g and E 1 g	32	**Rx** (2 months): ↓ DD (22 % → 9 %)
	TUNEL > 15 %		32	**Placebo group**: no effect on DD (22 % → 22 %)
Menezo et al. 2007	2 failed ICSI	Vits C and 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 et al. 2007	Male infert	Menevit (lycopene,	36	**Rx** (3 months): 39 % ICSI pregnancy rate,
	TUNEL > 25 %	vits C and E, zinc,		But no ↑ in embryo quality, no post-Rx DD
		Se, folate, garlic)	16	**Placebo group**: 16 % ICSI pregnancy rate
Gil-Villa et al. 2009	Pregn. loss	Vits C, E,	9	**Rx** (3 months): 6 (of 9) couples got pregnancy
	↑LPO or DFI	zinc, ß-carotene		**No control group**
Tunc et al. 2009	Male infert	Menevit (lycopene,	45	**Rx** (3 months): ↓ DD (22 % → 18 %)
	↑semen OS	vits C and E, zinc,		↓ROS production and ↑sperm protamination
		Se, folate, garlic)		**No control group**
Unselected infertile men				
Piomboni et al. 2008	Asthenosp.	Vits C and E, ß-glucan,	36	**Rx** (90 days): ↑motility and morph but not DD
	AO stain	papaya, lactoferrin	15	**Control group**: no effect
Kodama et al. 1997	Male infert	Vit C and E (200 mg),	14	**Rx** (2 months): ↓ in 8-OHdG (1.5 → 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, *OS* oxidative stress, *Rx* treatment, *ROS* reactive oxygen species, *Se* selenium, *TUNEL* terminal deoxynucleotidyl transferase dUTP nick end labeling, *vit* vitamin

of oxidative stress on sperm functional competence can be exaggerated by the in vitro sperm processing techniques (centrifugation, prolonged incubation) that usually precede assisted reproductive techniques (Chen et al. 2012; Twigg et al. 1998; Aitken and Baker 1995; Aitken and Clarkson 1988).

Role of In Vitro Antioxidants in Protecting Spermatozoa from Exogenous and Endogenous ROS

Attenuating the effects of exogenous ROS is clinically relevant as many of the semen samples from infertile men contain abnormal spermatozoa and leukocytes, and these cells have the potential to generate exogenous ROS (Aitken et al. 1995b). Antioxidants such as vitamin E, catalase, and glutathione have been shown to protect sperm motility from the effects of exogenous ROS (see Table 124.5) (Griveau et al. 1994). In contrast, superoxide dismutase is less effective in preventing the loss of motility due to exogenous oxidants (Griveau et al. 1994). Altogether, these data suggest that hydrogen peroxide (H_2O_2) is the most sperm-

Table 124.5 Role of in vitro antioxidants in protecting spermatozoa from the loss of motility and DNA damage due to exogenous ROS

Study	Exogenous ROS	Antioxidant supplement and results
Sperm motility		
de Lamirande and Gagnon 1992b	X + XO	Catalase protects spz from X + XO-induced loss of motility
		SOD, DTT, or GSH less effective in protecting spz motility from ROS
Griveau et al. 1994	X + XO	Catalase protects spz from X + XO-induced loss of motility
		SOD or mannitol ineffective in protecting spz motility from ROS
Sperm DNA		
Lopes et al. 1998	X + XO	GSH + hypotaurine protect spz from X + XO-induced DD
		Catalase protects spz from X + XO-induced DD
		n-Acetylcysteine protects spz from X + XO-induced DD
Potts et al. 2000	H_2O_2 + Fe + ADP	S. plasma (>60%v/v) lowers oxidative spz damage (↓DD, LPO)
Sierens et al. 2002	H_2O_2	Isoflavones and vits C and E protect spz from H_2O_2-induced DD
		(Isoflavones : genistein, equol). Dose effect noted
Russo et al. 2006	(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

toxic ROS. Antioxidants have also been shown to protect the sperm DNA from the effects of exogenous ROS (see Table 124.5) (Lopes et al. 1998; Potts et al. 2000; Russo et al. 2006; Sierens et al. 2002). This is highly relevant as sperm DNA damage may impact on reproductive outcomes after ARTs (Zini and Sigman 2009). Indeed, sperm DNA damage has been associated with reduced pregnancy rates with IUI and to a lesser extent with conventional IVF (Zini and Sigman 2009; Bungum et al. 2007; Collins et al. 2008).

Spermatozoa can be stimulated to generate ROS using a variety of agents (e.g., NADPH, estrogens), and this endogenous ROS production can potentially impair sperm function (Aitken et al. 1997). In contrast to the beneficial effect of antioxidants in protecting spermatozoa from exogenous ROS, antioxidants appear to be of limited value in protecting spermatozoa from endogenous ROS production (Twigg et al. 1998). Twigg et al. demonstrated that SOD, catalase, or both are ineffective, whereas albumin is effective in protecting spermatozoa from loss of motility due to endogenous ROS generation (Twigg et al. 1998). These studies stress the importance of using gentle semen processing protocols (e.g., low centrifugation force) so as to minimize the production and adverse impact of endogenous ROS.

Similarly, antioxidants appear to be of limited value in protecting the DNA of normal spermatozoa (with normal chromatin compaction) from endogenous ROS production (e.g., NADPH induced or centrifugation induced) (Twigg et al. 1998; Anderson et al. 2003; Cemeli et al. 2004; Dobrzynska et al. 2004). 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 (Muratori et al. 2000; Said et al. 2005). In support of these clinical observations, experimental (animal) studies suggest that the spermatozoa of infertile men may be more susceptible to oxidative injury in vitro but benefit more so from antioxidants than the spermatozoa of fertile men (Libman et al. 2010).

Summary

Oxidative stress plays an important role in the pathophysiology of male infertility. High levels of semen ROS can cause sperm dysfunction and reduced male reproductive potential. Studies have shown that dietary antioxidants generally have a beneficial effect on sperm function and male fertility potential. However, the mechanism of action of these oral antioxidants as well as the optimal type and dosage of antioxidant are unknown. The study of in vitro antioxidants is highly relevant in the era of assisted reproduction because of the susceptibility of human spermatozoa to oxidative injury and the vulnerability of these cells during semen processing. Most studies have demonstrated a beneficial effect of in vitro antioxidant supplements in protecting spermatozoa from exogenous oxidants but not from endogenous ROS.

Competing Financial Interests

Dr. Armand Zini is a shareholder in YAD technologies Inc. (a nutraceutical supplement company).

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