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DEBATE – continued

Is antioxidant therapy a promising strategy to improve human reproduction?

Are anti-oxidants useful in the treatment of male infertility?

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There are several lines of evidence that reactive oxygen species (ROS) produced by leukocytes and/or by spermatozoa from oligozoospermic men have a deleterious effect on sperm function (Aitken *et al*., 1989, 1994). Lipid peroxidation has been associated with midpiece abnormality, decreased sperm motility and loss of the capacity of the spermatozoon to undergo the acrosome reaction and to fertilize (Sukcharoen *et al*., 1996; Griveau and de Lannou 1997). ROS can be detected in 25–40% of semen samples from infertile men with idiopathic infertility and up to 96% of patients with spinal cord injury. Different enzymes and compounds present in seminal fluid have anti-oxidative activities: glutathione peroxidase/reductase, superoxide dismutase, pyruvate, taurine, hypotaurine, urate, and vitamins C and E (Lewis *et al*., 1997). Metal ions, e.g. copper and iron, may also modulate the antioxidant capacity of seminal plasma by changing the rate of ascorbate oxidation (Mendito *et al*., 1997).

Table I summarizes the results of the effects of antioxidants on sperm function and fertility. On the whole, the results of the in-vivo antioxidant trials reported to date are modest and certainly not better than controversial treatments of male infertility such as the treatment of prostatitis and varicocele or the avoidance of certain drugs (Martin-Du Pan *et al*., 1997). The only significant results on the pregnancy rate are observed in the non-randomized study of Suleiman *et al*. (1996) with no pregnancy in the placebo group and nine pregnancies in the treatment group (vitamin E 300 mg/day). However, a dose–response study has shown that the highest seminal concentration of tocopherol achieved after a treatment with 800 mg/day (0.96 μmol/l) was well below the antioxidant effective dose *in vitro* (10 mmol/l) (Moilanen and Hovatta, 1995). Therefore, the clinical efficiency of oral treatment with vitamin E seems doubtful with the usual posology. On the contrary, even small doses of vitamin C (200 mg), have been shown to increase the seminal level of ascorbate in smokers from 5.6 to 13.1 mg/dl, which is similar to the level achieved (16.1 mg/dl) after 1000 mg of vitamin C (Dawson *et al*., 1992). Smoking can decrease the level of seminal ascorbate which protects against oxidative DNA damage in human spermatozoa (Fraga *et al*., 1991). However, the deleterious effect of smoking on semen is still controversial (Cope and Mather, 1997). The administration of 200 and 1000 mg of vitamin C induced a non-significant increase of sperm concentration in smokers (Dawson *et al*., 1992).

In an unselected population of 152 and 86 infertile men with oligoasthenoteratozoospermia (OAT), 200 mg of vitamin C (which was introduced as a placebo) had no effect on sperm characteristics and induced a pregnancy rate similar to that observed after the administration of mesterolone and clomiphene (Abel *et al*., 1982; Hargreave *et al*., 1984). Logically, the antioxidants should be efficient only in OAT due to increased level of ROS. However, in infertile patients with a high level of oxidative DNA damage in spermatozoa, even the combination of vitamins C and E with glutathione induced only a slight increase in sperm concentration (Kodama *et al*., 1997).

Administration of vitamins C (350 mg/d) and E (250 mg) together *in vivo* were not able to prevent DNA sperm damage occurring after ejaculation (Hughes *et al*., 1997). Antioxidants would be interesting if they could be used to avoid assisted reproductive techniques in cases of severe male infertility or if they could improve the percentage of oocytes fertilized when in-vitro fertilization (IVF) is necessary. Due to the variability of oocyte fertilization from one cycle to the other, controlled studies are imperative. What is the in-vitro effect of antioxidants? Firstly, the beneficial role of a physiological level of ROS in human sperm activation and capacitation and sperm oocyte–fusion must be emphasized. When vitamins E and C are added to a sperm suspension, there is a decrease in the percentage of capacitated bull spermatozoa (O'Flaherty *et al*., 1997). On the other hand, the fertilizing ability of human spermatozoa is inversely related to sperm ROS production (Sukcharoen *et al*., 1996). The antioxidants could be useful in cases of excessive levels of ROS during sperm selection for IVF or intrauterine insemination (IUI). It has been shown that spermatozoa selected by Percoll gradient produce less ROS than those selected by centrifugation and swim-up (Aitken and Clarkson, 1988). The addition of antioxidants (e.g. superoxide dismutase) during centrifugation is able to prevent the fall in sperm motility and to improve the rate of both hyperactivation and the acrosome reaction (Griveau and Le Lannou, 1994). Similarly, *N*-acetyl-L-cysteine, a reducing substance, has been shown *in vitro* to improve sperm motility together with a decrease of ROS levels in infertile patients with high seminal level of ROS (Oeda *et al*., 1997). In another study, incubation during liquefaction and centrifugation with a solution containing glucose and glutathione increased recovery of motile

Table I. Clinical trials involving anti-oxidants and their relative effects on sperm parameters and pregnancy rates

Study	Medication	Duration	No. of cases	Sperm characteristics	Change observed in spermatozoa	Pregnancy (%)	Other effects reported
Moilanen and Hovatta (1995)	Vitamin E (600–1200 mg)	3 weeks	15	–	None	–	↑ seminal vitamin E <1 μmol after 1200 mg
Geva *et al.* (1996)	Vitamin E (200 mg)	2 months	15	↑ malondialdehyde production	None	–	↑ fertilization rate per cycle ↓ malondialdehyde
Kessopoulou *et al.* (1995)[a]	Vitamin E (300 mg)	3 months	30	$>5 \times 10^6$ spermatozoa	None	3	↑ zona binding
	Placebo					6	
Suleiman *et al.* (1996)	Vitamin E (300 mg)	6 months	52	all patients with	↑ motility in	17	No ↑ motility if patients
	Placebo		35	asthenozoospermia	Vitamin E group	0	had <15% motility
Abel *et al.* (1982)	Vitamin C (200 mg)	6 months	86	oligoastheno-	None	10	
	clomiphene (50 mg)		93	teratozoospermia		15	
Hargreave *et al.* (1984)	Vitamin C (200 mg)	9 months	152	oligoastheno-	None	18	
	mesterolone (2 × 50 mg)		176	teratozoospermia		19	
Dawson *et al.* (1992)	Vitamin C (200 mg)	4 weeks	25	None	↑ concentration	–	↑ of seminal ascorbate
	Vitamin C (1000 mg)		25	(smokers)	↑ concentration		and ↓ of non-specific sperm
	Placebo		25		none		agglutination
Lenzi *et al.* (1993)[a]	Glutathione (600 mg i.m.)	2 months	20	oligoastheno-teratozoospermia	↑ motility	–	
Kodama *et al.* (1997)	Vitamins C and E (200 mg)	2 months	14	oligoastheno-	↑ concentration	–	↑deoxyguanosine
	Glutathione (400 mg)		17	teratozoospermia			in spermatozoa

NS = not significant.
[a]Crossover trial.

spermatozoa (Parinaud *et al*., 1997). However, more studies are necessary to demonstrate the innocuity and the efficiency of these antioxidants on the pregnancy rate during IVF or IUI. Until now, even the beneficial effect of Percoll gradient preparation (low ROS) compared with centrifugation swim-up (high ROS) on the fertilization and the pregnancy rate is controversial (Griveau and Le Lannou, 1994).

In conclusion, even if we are fascinated by the role of ROS in sperm function we do not share the optimism of Tarín *et al.* (1998) on a potential role for antioxidant in the treatment of male infertility. We hope that further studies will contradict our opinion!

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