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INVITED REVIEW

The role of antioxidant therapy in the treatment of male infertility

ASHOK AGARWAL[1] & LUCKY H. SEKHON[2]

[1]*Center for Reproductive Medicine, Glickman Urological & Kidney Institute and Ob/Gyn & Women's Health Institute, Cleveland Clinic, Cleveland, Ohio, USA and* [2]*Royal College of Surgeons in Ireland, Dublin, Ireland*

Abstract
Oxidative stress contributes to defective spermatogenesis leading to male factor infertility. The aim of this study was to review the current literature on the effects of various antioxidants to improve fertilisation and pregnancy rates. The sources of literature were Pubmed and the Cochrane data base. Reviewing the current literature revealed that Carnitines and vitamin C and E have been clearly shown to be effective by many well-conducted studies and may be considered as a first line treatment. The efficacy of antioxidants, such as glutathione, selenium and coenzyme Q10 has been demonstrated by few, but well-performed studies, and may be considered second line treatment. There is, however, a need for further investigation with randomised controlled studies to confirm the efficacy and safety of antioxidant supplementation in the medical treatment of idiopathic male infertility as well as the need to determine the ideal dose of each compound to improve semen parameters, fertilisation rates and pregnancy outcomes.

Keywords: *Male infertility, spermatozoa, oxidative stress, antioxidant treatment*

Introduction

Infertility affects approximately 15% of all couples. Male factors contribute to approximately half of these cases, with no identifiable cause in 25% (Sharlip et al., 2002). 'Male factor' infertility is seen as an alteration in sperm concentration and/or motility and/or morphology in at least one sample of two sperm analyses, collected between 1 and 4 weeks apart (World Health Organization, 1999).

Free radicals contribute to the pathogenesis of male infertility (Figure 1). Free radicals are a group of highly reactive chemical molecules with one or more unpaired electrons that can oxidatively modify biomolecules they encounter. Reacting almost immediately with any substance in their surrounding area, they begin a chain reaction leading to cellular damage (Warren et al., 1987). Superoxide anion, hydroxyl radical and hydrogen peroxide are major reactive oxygen species (ROS) present in seminal plasma. Cells living under aerobic conditions require oxygen to support life; however, metabolites, such as ROS, can modify cell functions and endanger cell survival (Agarwal et al., 2003). Male germ cells at various stages of differentiation have the potential to generate ROS and low physiologic levels are needed to regulate sperm capacitation, acrosome reaction and sperm–oocyte fusion (Agarwal & Saleh, 2002; Agarwal et al., 2004). To maintain normal cell function, excess ROS must be continuously inactivated by seminal plasma antioxidants. These block the formation of new ROS or act as scavengers and remove ROS already generated. Natural antioxidant enzyme systems include catalase, glutathione peroxidase and superoxide dismutase (Baker et al., 1996). In healthy men, a delicate balance exists between physiological ROS and antioxidants in the male reproductive tract (Sikka et al., 1995).

Oxidative stress (OS) arises when excess free radicals overwhelm the antioxidant defence of the male reproductive tract (Sharma & Agarwal, 1996; Kemal Duru et al., 2000), damaging cells, tissues and organs (Gomez et al., 1998; Misro et al., 2004). Seminal OS correlates negatively with sperm concentration, motility and function-adversely affecting fusion events required for fertilisation (Aitken & Clarkson, 1987; Aitken et al., 1989; Sharma & Agarwal, 1996; Sikka, 2001). The polyunsaturated fatty acids of the sperm plasma membrane are susceptible to ROS damage as low concentrations of the scavenging enzymes are found in sperm cytoplasm (Saleh & Agarwal, 2002). ROS attack on

Correspondence: Ashok Agarwal, PhD, HCLD, Professor and Director, Center for Reproductive Medicine, Glickman Urological & Kidney Institute, Cleveland Clinic, 9500 Euclid Avenue, Desk A19.1, Cleveland, Ohio 44195, USA. Tel: +1-216-444-9485. Fax: +1-216-445-6049. E-mail: agarwaa@ccf.org

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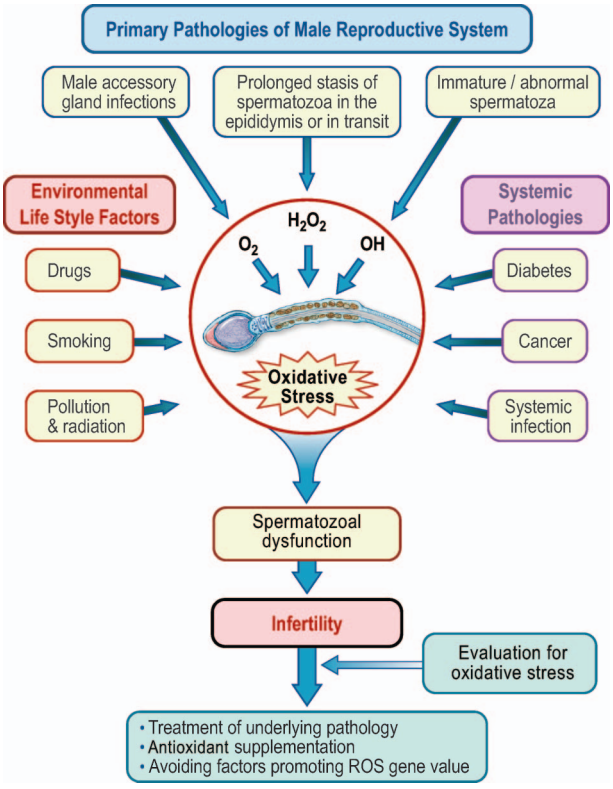

Figure 1. Factors contributing to oxidative stress-induced male infertility.

the cell membrane leads to a chain of chemical reactions called lipid peroxidation, which reduces membrane fluidity and the activity of membrane enzymes and ion channels, resulting in the inhibition of normal cellular mechanisms required for fertilisation. End products of lipid peroxidation, such as malondialdehyde (Sharma & Agarwal, 1996), are measured to estimate the extent of peroxidative damage.

Deoxyribonucleic acid bases and phosphodiester backbones of DNA can also experience peroxidative damage. Oxygen-radical induced caspase and endonuclease mechanistic pathways have been proposed as a potential cause of double-stranded DNA damage (Sakkas & Alvarez, 2010). Patients with high-seminal fluid OS were found to have sperm with multiple single and double DNA strand breaks (Twigg et al., 1998). Spermatozoal DNA is usually protected by its compact organisation and by antioxidants in the seminal plasma. Spermatozoa themselves are incapable of DNA repair and must depend on the oocyte for repair after fertilisation; however, knowledge concerning the process remains limited (Aitken et al., 2003). ROS exposure may result in DNA base modification, production of base-free sites, deletions, frame shifts, DNA cross-links and chromosomal aberrations (Duru et al., 2000; Agarwal & Said, 2003). A 8-hydroxy-2-deoxyguanosine (8-OHdG) is a biomarker that may determine the extent of ROS-induced DNA damage.

ROS may promote apoptosis: a process in which the body removes old and senescent cells (Agarwal et al., 2003), leading to decreased sperm concentration (Sikka, 2004). In a study from our centre (Wang et al., 2003), levels of caspases, proteases involved in apoptosis, correlated with ROS levels- implicating OS in increased apoptosis in mature spermatozoa. Our results showed that apoptosis could be induced in cell cultures with H_2O_2, further implicating ROS in the induction of apoptosis.

Antioxidants

Treatment strategies to reduce seminal OS levels may enhance natural conception and the outcome of assisted reproductive technologies. Antioxidants are the most important defence against free radical-induced infertility. Standard semen analysis and use of the sperm deformity index have been used to identify infertile males with high levels of ROS (Said et al., 2005). Seminal fluid OS levels can also be quantified either by direct methods, such as chemiluminescence assays, cytochrome-c and nitroblue tetrazolium reduction, flow cytometry, electron spin resonance spectroscopy, xylenol orange-based assay, and by indirect methods which measure the levels of biomarkers of OS, such as thiobarbituric acid-reactive substances, isoprostane, DNA damage and total antioxidant capacity. Measuring the degree of OS may identify those patients who could benefit from antioxidant supplementation (Fnu et al., 2008).

Several clinical trials have examined the potential of antioxidant supplementation to treat oxidative-stress-induced male factor infertility (Ross et al., 2010). The low cost and relatively low risk of toxicity of the following antioxidants is appealing to both patients and clinicians:

Carnitines

Carnitine is a water-soluble antioxidant mostly derived from the human diet that may play a role in sperm energy metabolism and provide the primary fuel for sperm motility. Spermatozoa exhibit increased L-carnitine and L-acetyl carnitine content during epididymal passage and acquisition of motility (Jeulin & Lewin, 1996). Carnitines enhance the cellular energetics in mitochondria by facilitating the entry and utilisation of free fatty acids within the mitochondria and also restore the phospholipid composition of mitochondrial membranes by decreasing fatty acid oxidation (Gattuccio et al., 2000; Vicari & Calogero, 2001; Lenzi et al., 2003). In addition, carnitines protect sperm DNA and cell membranes from ROS-induced damage and apoptosis

(Arduini, 1992; Lenzi et al., 2003, 2004; Cavallini et al., 2004). Patients with defective sperm motion parameters have been shown to have a reduced L-acetyl-carnitine/ L-carnitine ratio (Bartonelli et al., 1987). In a systematic review by Ross et al. (2101) carnitine oral supplementation showed improvements in both motility and concentration (Menchini et al., 1984; Bornman et al., 1989).

Preliminary, uncontrolled studies of infertile men (Moncada et al., 1992; Costa et al., 1994; Vitali et al., 1995) demonstrate a favourable effect of oral carnitine on sperm motion characteristics. A daily dose of 3 g given over a 3 (Vitali et al., 1995) or 4-month duration (Costa et al., 1994) significantly improved patients' sperm motility from pre-treatment levels. A daily dose of 4 g over 2 months increased progressive sperm motility in 15 of 20 patients. This effect was more pronounced in seven patients whose partners achieved pregnancy during treatment and follow-up. Carnitine's effects may depend on pre-treatment semen characteristics. Randomised controlled trials have demonstrated a daily dose of 2 g carnitine yielded the most significant improvement in subjects with lower baseline motility (Lenzi et al., 2003, 2004). However, Lenzi et al. (2003, 2004) failed to demonstrate any improvement in morphology, suggesting carnitine's effects are post-testicular. However, Cavallini et al. (2004) demonstrated improved morphology after 3 and 6 months of treatment. In all studies, the effects of carnitine were stable and no further improvement in sperm parameters was observed beyond the 3 to 6-month treatment period.

Vitamin E

The daily requirement of vitamin E (α-tocopherol) varies from 50 to 800 mg, depending on the intake of fruits, vegetables, tea or wine (National Academy of Sciences, 1989). Vitamin E is an important lipid-soluble antioxidant molecule in the cell membrane. It is thought to interrupt lipid peroxidation and enhance the activity of various antioxidants that scavenge free radicals generated during the univalent reduction of molecular oxygen and during normal activity of oxidative enzymes (Ehrenkranz, 1980; Palamanda & Kehrer, 1993). The results of *in vitro* experiments suggest that vitamin E may protect spermatozoa from oxidative damage and loss of motility as well as enhance the sperm performance in the hamster egg penetration assay (de Lamirande & Gagnon, 1992). Recent randomised control trials have reported vitamin E to be effective in treating infertile males with high-ROS levels (Kessopoulou et al., 1995; Suleiman et al., 1996; Ross et al., 2010). Vitamin E treatment decreased malondialdehyde (MDA) concentrations in spermatozoa down to normospermic levels, improving motility and the probability of achieving pregnancy (Suleiman et al., 1996). Vitamin E may also be added to cryoprotectants to protect spermatozoa from increased exposure to OS during cryopreservation and thawing procedures, which lead to reduced sperm motility (Dawson et al., 1987).

Vitamin C

Vitamin C (ascorbic acid) is a water-soluble ROS scavenger with high potency. It is found in concentrations 10-fold higher in seminal plasma than serum (Dawson et al., 1987; Jacob et al., 1992), protecting human spermatozoa against endogenous oxidative damage by neutralising hydroxyl, superoxide and hydrogen peroxide radicals and preventing sperm agglutination (Fraga et al., 1991). Significantly reduced concentrations are seen in semen samples with excess ROS (Lewis et al., 1997). Seminal plasma concentrations have been positively correlated with percentage of morphologically normal spermatozoa (Thiele et al., 1995). Vitamin C may have a dose-dependent effect on sperm quality. At a dose of 1000 μg/l, vitamin C positively influenced the motility of the spermatozoa. However, higher dosages may have damaging pro-oxidant effects which actually reduce sperm motility (Abel et al., 1983). Fraga et al. (1991) demonstrated a significant relationship between decreased seminal fluid vitamin C levels and increased 8-OHdg. Vitamin C supplementation may minimise endogenous oxidative DNA damage, thereby decreasing the risk of genetic defects, particularly in populations with low vitamin C levels, such as smokers.

The hydrophilicity and lipophilicity of vitamins C and E may act synergistically to protect against peroxidative attack on spermatozoa (Baker et al., 1996). Greco et al. (2005) demonstrated that combined supplementation significantly reduced the percentage of DNA-fragmented sperm. At follow-up, there were significantly increased rates of clinical pregnancy and implantation following intracytoplasmic sperm injection (ICSI). However, a randomised controlled double-blind study by Rolf et al. (1991) failed to show improvement in semen parameters, sperm survival or pregnancy rates in couples with male factor infertility after the administration of high-dose oral vitamin C and E for 56 days. Although several additional studies have not shown vitamin E and C to be effective (Giovenco et al., 1987; Kessopoulou et al., 1995; Moilanen & Hovatta, 1995; Rolf et al., 1999), further prospective controlled clinical studies should be carried out using selected patients with identified and known DNA damage for whom antioxidant treatment may be effective.

Selenium

Selenium (Se) may protect against oxidative sperm DNA damage and is required for normal testicular development, spermatogenesis, motility and function (Ursini et al., 1999). The precise mechanism by which Se eliminates OS is not well-established. Selenoenzymes, such as phospholipid hydroperoxide glutathione peroxidase (PHGPX) (Roveri et al., 1992) and the sperm capsular selenoprotein glutathione peroxidase may mediate its effects (Alvarez & Storey, 1984; Surai et al., 1998). Se deficiency leads to impaired motility, breakage of the spermatozoal mid-piece (Wallace et al., 1983, 1987) and increased morphological abnormalities, mostly affecting the sperm head (Watanabe & Endo, 1991; Noack-Filler et al., 1993). A significant correlation has been observed between sperm concentration and seminal plasma Se in patients with infertility (Bleau et al., 1984; Behne et al., 1988). However, a link between Se levels in semen or seminal plasma and sperm concentration or motility was not reproduced by other studies (Saaranen et al, 1987; Roy et al., 1990).

The effectiveness of combined treatment with Se and vitamin E has been studied since Vitamin E works synergistically with Se as an antiperoxidant (Maiorino et al., 1989; Burton & Traber, 1990). A prospective, uncontrolled study reported that combined treatment significantly increased motility and mean seminal plasma glutathione peroxidase (GSH-Px) activity. Although no improvements in sperm concentration or pregnancy rate were achieved, better sperm motion characteristics may have resulted from amplified antioxidant enzyme activity (Vezina et al., 1996). These results were confirmed by recent randomised controlled trial in which vitamin E and Se improved sperm motility and decreased lipid peroxidation markers (Kesker-Ammar et al., 2003).

Carotenoids

Carotenoids work synergistically with Se and vitamin E and have a recommended dietary allowances value of 1000 mg per day (National Academy of Sciences, 1989). In a recent double-blind randomised controlled trial, carotenoid compound Astaxanthin was administered at a dose of 16 mg/d for 3 months, resulting in increased total (54.5% compared to 10.5%) and per cycle (23.1% compared to 3.6%) pregnancy rates compared with a placebo group (Comhaire et al., 2005). Another carotenoid of interest is lycopene – naturally derived from fruits and vegetables. It has been found to have the highest ROS-quenching rate, with plasma levels higher than beta carotene (Klebanov et al., 1998). Lycopene is found in high concentrations in the testes and seminal plasma, with lower levels in infertile men. Gupta and Kumar (2002) reported that a twice a day dose of 200 mg for 3 months resulted in a statistically significant improvement in sperm concentration of 66% of patients and motility of 53%. Interestingly, those with very low baseline sperm concentration failed to exhibit significant response to therapy, whereas higher baseline concentrations were associated with significant improvement and resulted in six pregnancies in 26 patients. Therefore, larger randomised controlled trials are required to establish the patient subgroups that would derive the greatest benefit from lycopene therapy.

Glutathione and N-acetyl cysteine

Glutathione (GSH) is the most abundant reducing agent found in the body, protecting lipids, proteins and nucleic acids against oxidative damage. GSH combines with vitamin E and Se to form glutathione peroxidase. In a placebo-controlled, double-blind, cross-over trial, administration of 600 mg for 2 months by intramuscular injection in 20 infertile men significantly increased sperm motion characteristics, namely improved forward progression (Lenzi et al., 1993). GSH deficiency may render the mid-piece unstable, resulting in defective morphology and motility (Hansen & Deguchi, 1996; Ursini et al., 1999). *N*-acetyl cysteine (NAC) replenishes GSH while scavenging free radicals and reducing ROS production in human ejaculate (Gressier et al., 1994; Agarwal & Said, 2004). NAC plays an important role in germ cell survival in human seminiferous tubules *in vitro* (Erkkilä et al., 1998). Oeda et al. (1997) found that incubating semen samples with NAC for 20 min significantly decreased ROS levels and lead to improved sperm motility (Ross et al., 2010). An uncontrolled study by Comhaire et al.(2000) found that NAC improved sperm concentration and acrosome reaction, while reducing ROS and oxidation of sperm DNA; however, there was no effect on motility and morphology. A randomised controlled trial by Safarinejad and Safarinejad (2009) reported that NAC with Se has additive beneficial effects on mean sperm concentration and percent normal morphology. By the end of a 26-week treatment period, motility significantly improved in the combined treatment group and in those patients receiving Se alone, compared to placebo. Furthermore, combination treatment led to significantly better sperm parameters than treatment with only Se.

Pentoxifylline

Pentoxifylline is a competitive nonselective phosphodiesterase inhibitor that raises intracellular cAMP

and reduces inflammation by inhibiting TNF-α and leukotriene synthesis (Agarwal & Said, 2004). Pentoxifylline has been shown to decrease ROS production (Gavella & Lipovac, 1992; Gavella et al., 1991), preserve sperm motility *in vitro* (Pang et al., 1993) and improve semen parameters *in vivo* (Marrama et al., 1985; Yovich et al., 1990). Tesarik et al. (1992) demonstrated that pentoxifylline improved sperm motion characteristics, such as curvilinear velocity, path velocity and beat cross frequency but did not increase the percentage of motile spermatozoa in asthenospermic males. Okada et al. (1997) studied the effects of *in vitro* and *in vivo* pentoxifylline treatment on sperm motion parameters in male subjects whose spermatozoa produced detectable steady state levels of ROS. Treatment decreased ROS formation and preserved sperm motion parameters *in vitro*. Orally administered pentoxifylline had no effect at a low dosage, whereas a higher dosage increased sperm motility and some sperm motion parameters without altering sperm fertilising ability.

Trace metals

Adequate zinc and copper intake is needed to maintain the optimal functioning level of antioxidant enzymes, such as superoxide dismutase. The average daily intake in USA is 12.3 mg of zinc and 900 mg of copper per person. Seminal plasma zinc concentrations were shown to significantly differ between fertile and subfertile men (Chia et al., 2000). Zinc deficiency is associated with abnormal flagella showing marked fibrous sheath hypertrophy and hyperplasia, axonemal disruption and partial defects of the inner dynein arms of microtubular doublets, with distorted inner axonemal structure and a poorly formed or absent mid-piece (Omu et al., 2008). Prospective studies show an improvement of sperm concentration, (Hartoma et al., 1977; Tikkiwal et al., 1987; Omu et al., 1999), progressive motility (Ross et al., 2010), sperm integrity and pregnancy rates in subfertile males after zinc supplementation. A randomised controlled trial by Omu et al. (2008) reported zinc therapy in 11 infertile men to reduce seminal fluid MDA, TNF, apoptotic markers, antisperm antibodies and DNA fragmentation and enhance Zn-Cu-SOD activity and the expression of anti-inflammatory cytokine IL-4. Zinc therapy led to improved sperm parameters, although the improvements were not statistically significant.

Animal *in vivo* and *in vitro* studies have shown that zinc deficiency alters the absorption and metabolism of dietary folate (Ghishan et al., 1986; Quinn et al., 1990; Favier et al., 1993). A double-blind randomised controlled trial was conducted to assess whether zinc and folate supplementation work synergistically to improve semen quality (Wong et al., 2002). Folic acid was given at a daily dose of 5 mg, and zinc sulfate was given at a daily dose of 66 mg. Fertile and infertile subjects demonstrated a significant 74% increase in total normal sperm count and an increase of 4% in abnormal spermatozoa. However, whether the improvement in sperm concentration observed after administration of folic acid and zinc could increase the probability of achieving pregnancy remains to be established.

It is important to note that excessive intake of trace metals may have adverse, pro-oxidant effects as they can catalyse reactions that lead to the formation of ROS. An *in vitro* study showed that high concentrations of metal ions caused DNA strand breaks in salmon sperm (Lloyd et al., 1998). The ideal *in vivo* dosage required for an effective antioxidant effect in seminal plasma has yet to be determined. We recommend larger randomised, placebo-controlled studies on the efficacy and safety of trace metal supplementation.

Other

Menevit is an oral antioxidant supplement consisting of vitamin C, vitamin E, zinc, folic acid, lycopene, garlic oil and Se. In a prospective randomised double-blind trial involving 60 couples with severe male factor infertility, a daily oral Menevit tablet for 3 months before the partner's *in vitro* fertilisation cycle improved the viable pregnancy rate (38.5% of transferred embryos) compared to a placebo group (16% of transferred embryos) (Tremellen et al., 2007).

Coenzyme Q10 (CoQ-10) is found endogenously in the sperm mid-piece. CoQ-10 recycles vitamin E, controls its pro-oxidant capability and is involved in energy production (Lewin & Lavon, 1997). *In vitro* incubation of semen samples of infertile men with 50 mM of CoQ-10 significantly increased sperm motility. Oral supplementation with 60 mg of CoQ-10 in these infertile men was seen to improve fertilisation rate without effecting semen parameters (Thomas et al., 1997). Oral CoQ-10 administration has been shown to inhibit hydrogen peroxide formation in seminal fluid (Alleva et al., 1997). Catalase is another antioxidant which should be further investigated for its ability to detoxify both intracellular and extracellular hydrogen peroxide to water and oxygen (Baker et al., 1996). Compounds which regenerate antioxidant stores through redox cycling, such as a-lipoic acid, may be useful to potentiate the effects of vitamin C and E and GSH (Biewenga et al., 1997).

Table I provides an overview of studies of antioxidant compounds in the treatment of male factor infertility.

Table I. Overview of studies of antioxidant compounds in the treatment of male factor infertility.

Compound	Positive effect	Negative/no effect
Carnitine		
	Costa et al., 1994 Vitali et al., 1995 Moncada et al., 1992 Lenzi et al., 2003 Lenzi et al., 2004 Cavallini et al., 2004	
Vitamin E		
	de Lamirande & Gagnon, 1992 Suleiman et al., 1996 Dawson et al., 1987	
Vitamin E and C		
	Greco et al., 2005	Kessopoulou et al., 1995 Rolf et al., 1999 Giovenco et al., 1987 Moilanen & Hovatta, 1995
Selenium and Vitamin C		
	Vezina et al., 1996 Kesker-Ammar et al., 2003	
Carotenoids		
	Comhaire et al., 2005 Gupta & Kumar, 2002	
Glutathione and *N*-acetyl cysteine		
	Lenzi et al., 1993 Oeda et al., 1997 Comhaire et al., 2000	
N-acetyl cysteine and selenium		
	Safainejad et al., 2009	
Pentoxifylline		
	Gavella & Lipovac, 1992 Gavella et al., 1991 Pang et al., 1993 Marrama et al., 1985 Yovich et al., 1990 Tesarik et al., 1992 Okada et al., 1997	
Zinc		
	Omu et al., 1999 Hartoma et al., 1977 Tikkiwal et al., 1987 Omu et al., 2008	
Zinc and Folate		
	Wong et al., 2002	
Menevit		
	Tremellen et al., 2007	
Co-Enzyme Q10		
	Thomas et al., 19977 Alleva et al., 1997	

Conclusion

Infertile men are likely to be prescribed a number of empirical therapies. There is a rationale to support the use of antioxidants in infertile male patients with high OS status. However, the amount of scientifically acceptable evidence clearly showing their benefit in controlled human studies is sparse. For a drug to be considered effective, it should improve sperm parameters and pregnancy rates in at least one blind, prospective, placebo-controlled trial. The existing evidence supports the use of systemic antioxidants for the management of selective cases of male infertility as well as *in vitro* supplements during various sperm preparation techniques. A definitive conclusion, however, cannot be drawn as much of the literature is based on heterogeneous studies regarding a disease of multifactorial aetiology. The precise dose and duration of antioxidant therapy raises many questions as the level of ROS required for physiological processes and the level at which these compounds have toxic, pro-oxidant effects is not known. Some studies were unable to show the effectiveness of antioxidants on sperm parameters or fertility. This may be due to insufficient study duration or inadequate sample sizes. Future multicenter randomised control studies with larger samples will allow for better insight regarding the efficacy and safety of antioxidant supplementation in the treatment of male infertility.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

References

Abel, B. J., Carswell, G., & Elton, R. (1983). Randomised trial of clomiphene citrate treatment and vitamin C for male infertility. *British Journal of Urology*, *54*, 780–784.

Agarwal, A., Nallella, K. P., Allamaneni, S. S., & Said, T. M. (2004). Role of antioxidants in treatment of male infertility: an overview of the literature. *Reproductive Biomedicine Online*, *8*, 616–627.

Agarwal, A., & Said, T. M. (2003). Role of sperm chromatin abnormalities and DNA damage in male infertility. *Human Reproduction Update*, *9*, 331–345.

Agarwal, A., & Said, T. M. (2004). Carnitines and male infertility. *Reproductive Biomedicine Online*, *8*, 376–384.

Agarwal, A., & Saleh, R. A. (2002). Role of oxidants in male infertility: rationale, significance, and treatment. *Urologic Clinics of North America*, *29*, 817–827.

Agarwal, A., Saleh, R. A., & Bedaiwy, M. A. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility*, *79*, 829–843.

Aitken, R. J., Baker, M. A., & Sawyer, D. (2003). Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. *Reproductive Biomedicine Online*, *7*, 65–70.

Aitken, R. J., & Clarkson, J. S. (1987). Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *Journal of Reproduction and Fertility*, *81*, 459–469.

Aitken, R. J., Clarkson, J. S., & Fishel, S. (1989). Generation of reactive oxygen species, lipid peroxidation, and human sperm function. *Biology of Reproduction*, *41*, 183–197.

Alleva, R., Scararmucci, A., Mantero, F., Bompadre, S., Leoni, L., & Littarru, G. P. (1997). The protective role of ubiquinol-10 against formation of lipid hydroperoxides in human seminal fluid. *Molecular Aspects of Medicine*, *18*(Suppl), S221–S228.

Alvarez, J. G., & Storey, B. T. (1984). Lipid peroxidation and the reactions of superoxide and hydrogen peroxide in mouse spermatozoa. *Biology of Reproduction*, *30*, 833–841.

Arduini, A. (1992). Carnitine and its acyl esters as secondary antioxidants? *American Heart Journal*, *123*, 1726–1727.

Baker, H. W., Brindle, J., Irvine, D. S., & Aitken, R. J. (1996). Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leukocytes. *Fertility and Sterility*, *65*, 411–419.

Bartonelli, M., Canale, D., Izzo, P. L., Giorgi, P. M., Meschini, P., & Menchini-Fabris, G. F. (1987). L-carnitine and acetylcarnitine in human sperm with normal and reduced motility. *Acta Europaea Fertilitatis*, *18*, 29–31.

Behne, D., Gebner, H., Wolters, G., & Brotherton, J. (1988). Selenium, rubidium and zinc in human semen and semen fractions. *International Journal of Andrology*, *11*, 415–423.

Biewenga, G. P., Haenen, G. R., & Bast, A. (1997). The pharmacology of the antioxidant lipoic acid. *General Pharmacology*, *29*, 315–331.

Bleau, G., Lemarbre, J., Faucher, G., Roberts, K. D., & Chapdelaine, A. (1984). Semen selenium and human fertility. *Fertility and Sterility*, *42*, 890–894.

Bornman, M. S., du Toit, D., Otto, B., Muller, I. L., Hurter, P., & du Plessis, D. J. (1989). Seminal carnitine, epididymal function and spermatozoal motility. *South African Medical Journal*, *75*, 20–21.

Burton, G. W., & Traber, M. G. (1990). Vitamin E: antioxidant activity, biokinetics, and bioavailability. *Annual Review of Nutrition*, *10*, 357–382.

Cavallini, G., Ferraretti, A. P., Gianaroli, L., Biagiotti, G., & Vitali, G. (2004). Cinnoxicam and L-carnitine/acetyl-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. *Journal of Andrology*, *25*, 761–770.

Chia, S. E., Ong, C. N., Chua, L. H., Ho, L. M., & Tay, S. K. (2000). Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. *Journal of Andrology*, *21*, 53–57.

Comhaire, F. H., Christophe, A. B., Zalata, A. A., Dhooge, W. S., Mahmoud, A. M., & Depuydt, C. E. (2000). The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *6*, 159–165.

Comhaire, F. H., El Garem, Y., Mahmoud, A., Eertmans, F., & Schoonjans, F. (2005). Combined conventional antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial. *Asian Journal of Andrology*, *7*, 257–262.

Costa, M., Canale, D., Filicori, M., D'Iddio, S., & Lenzi, A. (1994). L-carnitine in idiopathic asthenozoospermia: a multicenter study. *Andrologia*, *26*, 155–159.

Dawson, E. B., Harris, W. A., Rankin, W. E., Charpentier, L. A., & McGanity, W. J. (1987). Effect of ascorbic acid on male fertility. *Annals of the New York Academy of Sciences*, *498*, 312–323.

de Lamirande, E., & Gagnon, C. (1992). Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes. *Journal of Andrology*, *13*, 368–378.

Duru, N., Morshedi, M., & Oehninger, S. (2000). Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertility and Sterility*, *74*, 1200–1207.

Ehrenkranz, R. (1980). Vitamin E and the neonate. *American Journal of Diseases of Children*, *134*, 1157–1168.

Erkkilä, K., Hirvonen, V., Wuokko, E., Parvinen, M., & Dunkel, L. (1998). *N*-acetyl-L cysteine inhibits apoptosis in human male germ cells in vitro. *Journal of Clinical Endocrinology and Metabolism*, *83*, 2523–2531.

Favier, M., Faure, P., Roussel, A. M., Coudray, C., Blache, D., & Favier, A. (1993). Zinc deficiency and dietary folate metabolism in pregnant rats. *Journal of Trace Elements and Electrolytes in Health and Disease*, *7*, 19–24.

Fnu, D., Cocuzza, M., & Agarwal, A. (2008). Should seminal oxidative stress measurement be offered routinely to men presenting for infertility evaluation? *Endocrine Practice*, *14*, 484–491.

Fraga, C. G., Motchnik, P. A., Shigenaga, M. K., Helbock, H. J., Jacob, R. A., & Ames, B. N. (1991). Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proceedings of the National Academy of Sciences USA*, *88*, 11003–11006.

Gattuccio, F., De Rose, A. F., & Latteri, M. A., Eds. (2000). Varicocele. Palermo, Italy: Cofese Editore.

Gavella, M., & Lipovac, V. (1992). Pentoxifylline-mediated reduction of superoxide anion production by human spermatozoa. *Andrologia*, *24*, 37–39.

Gavella, M., Lipovac, V., & Marotti, T. (1991). Effect of pentoxifylline on superoxide anion production by sperm. *International Journal of Andrology*, *14*, 320–327.

Ghishan, F. K., Said, H. M., Wilson, P. C., Murrell, J. E., & Greene, H. L. (1986). Intestinal transport of zinc and folic acid: a mutual inhibitory effect. *American Journal of Clinical Nutrition*, *43*, 258–262.

Giovenco, P., Amodei, M., Barbieri, C., Fasani, R., Carosi, M., & Dondero, F. (1987). Effects of kallikrein on the male reproductive system and its use in the treatment of idiopathic oligozoospermia with impaired motility. *Andrologia*, *19*, 238–241.

Gomez, E., Irvine, D. S., & Aitken, R. J. (1998). Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function. *International Journal of Andrology*, *21*, 81–94.

Greco, E., Iacobelli, M., Rienzi, L., Ubaidi, F., Ferrero, S., & Tesarik, J. (2005). Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *Journal of Andrology*, *26*, 349–353.

Gressier, B., Cabanis, A., Lebeque, S., Brunet, C., Dine, T., Luyckx, M., et al. (1994). Decrease of hypochlorous acid and hydroxyl radical generated by stimulating human neutrophils: comparison in vitro of some thiol-containing drugs. *Methods and Findings in Experimental Clinical Pharmacology*, *16*, 9–13.

Gupta, N. P., & Kumar, R. (2002). Lycopene therapy in idiopathic male infertility – a preliminary report. *International Urology and Nephrology*, *34*, 369–372.

Hansen, J. C., & Deguchi, Y. (1996). Selenium and fertility in animals and man – a review. *Acta Veterinaria Scandinavica*, *37*, 19–30.

Hartoma, T. R., Nahoul, K., & Netter, A. (1977). Zinc, plasma androgens and male sterility [letter]. *Lancet*, *2*, 1125–1126.

Jacob, R. A., Pianalto, F. S., & Agee, R. E. (1992). Cellular ascorbate depletion in healthy men. *Journal of Nutrition*, *122*, 1111–1118.

Jeulin, C., & Lewin, L. M. (1996). Role of free L-carnitine and acetyl- L -carnitine in post-gonadal maturation of mammalian spermatozoa. *Human Reproduction Update*, *2*, 87–102.

Kemal Duru, N., Morshedi, M., & Oehninger, S. (2000). Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertility and Sterility*, *74*, 1200–1207.

Kesker-Ammar, L., Feki Chacroun, N., Rebai, T., Sahnoun, Z., Ghozzi, H., Hammami, S., et al. (2003). Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Archives of Andrology*, *49*, 83–94.

Kessopoulou, E., Powers, H. J., Sharma, K. K., Pearson, M. J., Russell, J. M., Cooke, I. D., 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. *Fertility and Sterility*, *64*, 825–831.

Klebanov, G. I., Kapitanov, A. B., Teselkin YuO, Babenkova, I. V., Zhambalova, B. A., Lyubitsky, O. B., et al. (1998). The antioxidant properties of lycopene. *Membrane Cell Biology*, *12*, 287–300.

Lenzi, A., Culasso, F., Gandini, L., Lombardo, F., & Dondero, F. (1993). Placebo-controlled, double-blind, cross-over trial of glutathione therapy in male infertility. *Human Reproduction*, *8*, 1657–1662.

Lenzi, A., Lombardo, F., Sgrò, P., Salacone, P., Caponnecchia, L., & Dondero, F. (2003). Use of carnitine therapy in selected cases of male factor infertility: a double blind cross over trial. *Fertility and Sterility*, *79*, 292–300.

Lenzi, A., Sgrò, P., Salacone, P., Paoli, D., Gilio, B., Lombardo, F., et al. (2004). A placebo-controlled double-blind randomized trial of the use of combined L-carnitine and L-acetyl-carnitine treatment in men with asthenozoospermia. *Fertility and Sterility*, *81*, 1578–1584.

Lewin, A., & Lavon, H. (1997). The effect of coenzyme Q10 on sperm motility and function. *Molecular Aspects of Medicine*, *18*(Suppl), S213–S219.

Lewis, S. E., Sterling, E. S., Young, I. S., & Thompson, W. (1997). Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. *Fertility and Sterility*, *67*, 142–147.

Lloyd, D. R., Carmichael, P. L., & Phillips, D. H. (1998). Comparison of the formation of 8-hydroxy-29-deoxyguanosine and single- and double-strand breaks in DNA mediated by fenton reactions. *Chemical Research Toxicology*, *11*, 420–427.

Maiorino, M., Coassin, M., Roveri, A., & Ursini, F. (1989). Microsomal lipid peroxidation: effect of vitamin E and its functional interaction with phospholipid hydroperoxide glutathione peroxidase. *Lipids*, *24*, 721–726.

Marrama, P., Baraghini, G. F., Carani, C., Celani, M. F., Giovenco, P., Grandi, F., & Montanini, V. (1985). Further studies on the effect of pentoxifylline on sperm count and sperm motility in patients with idiopathic oligo-asthenozoospermia. *Andrologia*, *17*, 612–616.

Menchini Fabris, G. F., Canale, D., Izzo, P. L., Olivieri, L., & Bartonelli, M. (1984). Free L-carnitine in human semen: its variability in different andrologic pathologies. *Fertility and Sterility*, *42*, 263–267.

Misro, M. M., Choudhury, L., Upreti, K., Gautam, D., Chaki, S. P., Mahajan, A. S., & Babbar, R. (2004). Use of hydrogen peroxide to assess the sperm susceptibility to oxidative stress in subjects presenting a normal semen profile. *International Journal of Andrology*, *27*, 82–87.

Moilanen, J., & Hovatta, O. (1995). Excretion of alpha-tocopherol into human seminal plasma after oral administration. *Andrologia*, *27*, 133–136.

Moncada, M. L., Vicari, E., Cimino, C., Calogero, A. E., Mongioi, A., & D'Agata, R. (1992). Effect of acetylcarnitine treatmetn in oligoasthenospermic patients. *Acta Europaea Fertilitatis*, *23*, 221–224.

National Academy of Sciences. (1989). *Recommended dietary allowances, 10th ed.* United States of America, National Academy Press.

Noack-Fille, G., De Beer, C., & Sweibert, H. (1993). Cadmium, lead, selenium, and zinc in semen of occupationally unexposed men. *Andrologia*, *25*, 7–12.

Oeda, T., Henkel, R., Ohmori, H., & Schill, W. B. (1997). Scavenging effect of *N*-acetyl-L cysteine against reactive oxygen species in human semen: a possible therapeutic modality for male factor infertility? *Andrologia*, *29*, 125–131.

Okada, H., Tatsumi, N., Kanzaki, M., Fujisawa, M., Arakawa, S., & Kamidono, S. (1997). Formation of reactive oxygen species by spermatozoa from asthenospermic patients: response to treatment with pentoxifylline. *The Journal of Urology*, *157*, 2140–2146.

Omu, A. E., Al-Azemi, M. K., Kehinde, E. O., Anim, J. T,. Oriowo, M. A., & Mathew, T. C. (2008). Indications of the mechanisms envolved in improved sperm parameters by zinc therapy. *Medical Principles and Practice*, *17*, 108–116.

Omu, A. E., Al-Qattan, F., Abdulhadi, F., & Fernandes, S. (1999). Seminal immune response in infertile men with leukocytospermia: effect on antioxidant activity. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, *79*, 179–184.

Palamanda, J. R., & Kehrer, J. R. (1993). Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation by glutathione. *Lipids*, *28*, 427–43l.

Pang, S. C., Chan, P. J., & Lu, A. (1993). Effect of pentoxifylline on sperm motility and hyperactivation in normozoospermic and normokinetic semen. *Fertility and Sterility*, *60*, 336.

Quinn, P. B., Cremin, F. M., O'Sullivan, V. R., Hewedi, F. M., & Bond, R. J. (1990). The influence of dietary folate supplementation on the incidence of teratogenesis in zinc-deficient rats. *British Journal of Nutrition*, *64*, 233–243.

Rolf, C., Cooper, T. G., Yeung, C. H., & Nieschlag, E. (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. *Human Reproduction*, *14*, 1028–1033.

Ross, C., Morriss, A., Khairy, M., Khalaf, Y., Braude, P., Coomarasamy, A., et al. (2010). A systematic review of the effect of oral antioxidants on male infertility. *Reproductive Biomedicine Online*, *20*, 711–723.

Roveri, A., Casasco, A., Maiorino, M., Dalan, P., Calligaro, A., & Ursini, F. (1992). Phospholipid hydroperoxide glutathione peroxidase of rat testis: gonadotropin dependence and immunocytochemical identification. *Journal of Biological Chemistry*, *267*, 6142–6146.

Roy, A. C., Karunanithy, R., & Ratnam, S. S. (1990). Lack of correlation of selenium level in human semen with sperm count/motility. *Archives of Andrology*, *25*, 59–62.

Saaranen, M., Suistomaa, U., Kantola, M., Saarikoski, S., & Vanha-Perttula, T. (1987). Lead, magnesium, selenium and zinc in human seminal fluid: comparison with semen parameters and fertility. *Human Reproduction*, *2*, 475–479.

Safarinejad, M. R., & Safarinejad, S. (2009). Efficacy of selenium and/or *N*-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. *Journal of Urology*, *181*, 741–751.

Said, T. M., Aziz, N., Sharma, R. K., Lewis-Jones, I., Thomas, A.J. Jr., & Agarwal, A. (2005). Novel association between sperm deformity index and oxidative stress-induced DNA damage in infertile male patients. *Asian Journal of Andrology*, *7*, 121–126.

Sakkas, D., & Alvarez, J. (2010). Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. *Fertility and Sterility*, *93*, 1027–1036.

Saleh, R. A., & Agarwal, A. (2002). Oxidative stress and male infertility: from research bench to clinical practice. *Journal of Andrology*, *23*, 737–752.

Sharlip, I. D., Jarow, J. P., Belker, A. M., Lipshultz, L. I., Sigman, M., Thomas, A. J., et al. (2002). Best practice policies for male infertility. *Fertility and Sterility*, *77*, 873–882.

Sharma, R. K., & Agarwal, A. (1996). Role of reactive oxygen species in male infertility. *Urology*, *48*, 835–850.

Sikka, S. C. (2001). Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*, *8*, 851–862.

Sikka, S. C. (2004). Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. *Journal of Andrology*, *25*, 5–18.

Sikka, S. C., Rajasekaran, M., & Hellstrom, W. J. (1995). Role of oxidative stress and antioxidants in male infertility. *Journal of Andrology*, *16*, 464–468.

Suleiman, S. A., Ali, M. E., Zaki, Z. M., el-Malik, E. M., & Nasr, M. A. (1996). Lipid peroxidation and human sperm motility: protective role of vitamin E. *Journal of Andrology*, *17*, 530–537.

Surai, P. F., Blesbois, E., & Grasseau, I. (1998). Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. *Comparative Biochemistry and Physiology*, *120*, 527–533.

Tesarik, J., Thebault, A., & Testart, J. (1992). Effect of pentoxifylline on sperm movement characteristics in normozoospermic and asthenozoospermic specimens. *Human Reproduction*, *7*, 1257–1263.

Thiele, J. J., Friesleben, H. J., Fuchs, J., & Ochsendorf, F. R. (1995). Ascorbic acid and urate in human seminal plasma: determination and interrelationships with chemiluminescence in washed semen. *Human Reproduction*, *10*, 110–115.

Thomas, S. R., Neuzil, J., & Stocker, R. (1997). Inhibition of LDL oxidation by ubiquinol-10. A protective mechanism for coenzyme Q in atherogenesis? *Molecular Aspects of Medicine*, *18*(Suppl), S85–S103.

Tikkiwal, M., Ajmera, R. L., & Mathur, N. K. (1987). Effect of zinc administration on seminal zinc and fertility of oligospermic males. *Indian Journal of Physiology and Pharmacology*, *31*, 30–34.

Tremellen, K., Miari, G., Froiland, D., & Thompson, J. (2007). A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *47*, 216–221.

Twigg, J. P., Irvine, D. S., & Aitken, R. J. (1998). Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. *Human Reproduction*, *13*, 1864–1871.

Ursini, F., Heim, S., Kiess, M., Maiorino, M., Roveri, A., Wissing, J., et al. (1999). Dual function of the selenoprotein PHGPx during sperm maturation. *Science*, *285*, 1393–1396.

Vezina, D., Mauffette, F., Roberts, K. D., & Bleau, G. (1996). Selenium-vitamin E supplementation in infertile men. *Biological Trace Element Research*, *53*, 65–83.

Vicari, E., & Calogero, A. E. (2001). Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis. *Human Reproduction*, *16*, 2338–2342.

Vitali, G., Parente, R., & Melotti, C. (1995). Carnitine supplementation in human idiopathic asthenospermia: clinical results. *Drugs Under Experimental and Clinical Research*, *4*, 157–159.

Wallace, E., Calvin, H. I., Ploetz, K., & Cooper, G. W. (1987). Functional and developmental studies on the role of selenium in spermatogenesis. In Combs, G. F. Jr., Spallholz, J. E., Levander, O. A., Oldfield, J. E., (Eds), *Selenium in biology and medicine, part A.* New York: AVI, p 181–196.

Wallace, E., Cooper, G. W., & Calvin, H. I. (1983). Effects of selenium deficiency on the shape and arrangement of rodent sperm mitochondria. *Gamete Research*, *4*, 389–399.

Wang, X., Sharma, R. K., Sikka, S. C., Thomas, A. J., Falcone, T., & Agarwal A. (2003). Oxidative stress is associated with increased apoptosis leading to spermatozoa DNA damage in patients with male factor infertility. *Fertility and Sterility*, *80*, 531–535.

Warren, J. S., Johnson, K. J., & Ward, P. A. (1987). Oxygen radicals in cell injury and cell death. *Pathology and Immunopathology Research*, *6*, 301–315.

Watanabe, T., & Endo, A. (1991). Effects of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice. *Mutation Research*, *262*, 93–99.

Wong, W. Y., Merkus, H. M., Thomas, C. M., Menkveld, R., Zielhuis, G. A., & Steegers-Theunissen, R. P. (2002). Effects of folic acid and zinc sulphate on male factor subfertility: a double-blinded, randomized, placebo-controlled trial. *Fertility and Sterility*, *77*, 491–498.

World Health Organization. (1999). *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction, 4th ed.* Cambridge: Cambridge University Press, p.1–86.

Yovich, J. M., Edirisinghe, W. R., Cummins, J. M., & Yovich, J. L. (1990). Influence of pentoxifylline in severe male factor infertility. *Fertility and Sterility*, *53*, 715–722.