

A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment

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Abstract

Background: Evidence has accumulated supporting the role of reactive oxygen species (ROS) in the pathogenesis of sperm dysfunction among men with infertility. Damage to sperm DNA by ROS can lead to failure of conception, miscarriage or potentially even childhood cancer. The objective of this study was to examine the effect of male antioxidant treatment on embryo quality and pregnancy outcome during *in vitro* fertilisation-intracytoplasmic sperm injection (IVF-ICSI) treatment.

Methods: Sixty couples with severe male factor infertility were enrolled in a prospective randomised double-blind placebo-controlled trial. Male participants were randomly assigned to take either one capsule per day of the Menevit antioxidant or an identical in appearance placebo for three months prior to their partner's IVF cycle. The primary outcome was cleavage stage embryo quality and the secondary outcomes were oocyte fertilisation rate, pregnancy rates and treatment side-effects. Approval by the local Human Research Ethics Committee was obtained prior to the commencement of this study.

Results: The antioxidant group recorded a statistically significant improvement in viable pregnancy rate (38.5% of transferred embryos resulting in a viable fetus at 13 weeks gestation) compared to the control group (16% viable pregnancy). No significant changes in oocyte fertilisation rate or embryo quality were detected between the antioxidant and the placebo groups. Side-effects on the Menevit antioxidant were rare (8%) and mild in nature.

Conclusions: The Menevit antioxidant appears to be a useful ancillary treatment that significantly improves pregnancy rates in couples undergoing IVF-ICSI treatment for severe male factor infertility.

Key words: antioxidant, IVF-ICSI, oxidative stress, spermatozoa.

Introduction

Male infertility affects one man in 20, contributes to half of all infertility problems, and is the underlying solitary reason for access to *in vitro* fertilisation (IVF) treatment in 30% of IVF cycles conducted within Australia and New Zealand.^{1,2} Traditionally, the treatment of male infertility has been based on the use of 'mechanical' techniques such as IVF-intracytoplasmic sperm injection (ICSI) to help bypass defects in sperm function, rather than trying to ameliorate the underlying cause. However, now that it is recognised that free radical damage to sperm is a major cause of male infertility, interest in the use of antioxidants to treat male infertility is growing.

Free radicals, otherwise known as reactive oxygen species (ROS), are a group of highly reactive oxygen-based molecules that have one or more unpaired electrons capable of oxidising adjacent biomolecules. Spermatozoa require small amounts of ROS to initiate the acrosomal reaction and fusion with the oocyte membrane during fertilisation.^{3,4} However, when levels

of ROS within semen become excessive, damage to the sperm membrane and DNA can occur. Studies suggest that 30–80% of unselected infertile men have oxidative stress-related infertility.⁵

Within semen, there are two principal sources of ROS production: leucocytes and immature sperm.⁶ Conditions such as male genital tract infection, varicocele, smoking and exposure to environmental pollutants are all linked with excess leucocyte ROS production.⁵ Immature spermatozoa produce significant amounts of ROS as they contain excess cytoplasmic enzymes involved in ROS generation.⁶ Therefore, men with low normal sperm morphology have been identified

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Table 1 Study capsule components

1. Menevit active capsule
Lycopene 6 mg
Vitamin E 400 IU
Vitamin C 100 mg
Zinc 25 mg
Selenium 26 µgm
Folate 0.5 mg
Garlic 1000 mg
Palm oil (vehicle)
2. Placebo capsule
Palm oil

as having excessive seminal ROS production.^{7,8} Finally, in order to balance the potentially harmful effects of ROS, seminal plasma contains numerous antioxidants.⁶ Infertile men have been identified as having lower levels of antioxidants in their semen compared to fertile controls,^{6,9,10} thereby exposing these men to an increased risk of ROS damage to their sperm.

To date, over 20 studies have been published investigating the effects of oral antioxidant supplementation on sperm function.¹¹ Unfortunately, only two studies actually examine pregnancy outcome in a scientifically rigorous randomised placebo-controlled manner. Suleiman *et al.*¹² reported that treatment with oral vitamin E resulted in a significant fall in ROS damage to sperm and an improvement in spontaneous pregnancy rates during the next six months (21% pregnant rate in the vitamin E group vs 0% placebo). Conversely, Rolf *et al.*¹³ did not report any improvement in pregnancy outcome from two months treatment with a combination of vitamin C and vitamin E. Finally, Greco *et al.*¹⁴ reported in a placebo-controlled randomised control trial (RCT) that two months treatment with vitamin C and vitamin E could result in a significant reduction in free radical-related sperm DNA fragmentation. While a reduction in sperm DNA fragmentation may boost pregnancy rates, unfortunately, this was not examined in this study. Therefore, the role of antioxidants in boosting pregnancy rates is presently still under debate.

While several antioxidants are available 'over the counter', none are specifically designed to combat male infertility. The contents of the Menevit (Bayer, Sydney, Australia) antioxidant (Table 1) were specifically formulated to reduce sperm oxidative stress-related damage through three key mechanisms. Firstly, vitamins C and E, selenium, garlic and lycopene have the capacity to neutralise free radicals already produced by sperm or leucocytes.^{11,15–18} Secondly, lycopene¹⁶ and garlic¹⁹ have potent anti-inflammatory activity, thereby reducing seminal leucocytes production of free radicals. Finally, zinc, folate and selenium play a role in augmenting protamine packaging of sperm DNA,^{20–22} thereby protecting the DNA from oxidative stress. We therefore hypothesised that the Menevit antioxidant would reduce oxidative sperm damage leading to an improvement in embryo quality and pregnancy rates. The current prospective randomised placebo-controlled trial was designed to test this hypothesis.

Methods

Participants for this study were recruited from couples undergoing IVF-ICSI treatment at a single ART unit (Repromed, Dulwich, SA, Australia). To be eligible for enrolment, men were required to exhibit both likely oxidative stress (poor sperm morphology, motility or low membrane integrity) and a significant level of sperm DNA fragmentation (> 25% TUNEL positive). All semen samples were produced by masturbation after a period of three to five days abstinence and analysed for sperm count, motility, morphology and membrane integrity (Hypo-Osmolar Swelling test) as per WHO guidelines.²³ The remaining semen sample was frozen for later analysis of DNA fragmentation by microscopic TUNEL assay according to the protocol of Lopes *et al.*²⁴

All participants were booked to have a cycle of IVF-ICSI treatment within three months of enrolment. Female partners with diminished ovarian reserve (less than five oocytes in a prior IVF cycle or elevated early follicular phase FSH result) or those older than 39 years of age were excluded from the trial. Recruitment commenced in December 2004 and was completed by May 2006. The study was approved by the Women's and Children's Hospital Research Ethics Committee and registered with the NIH public trials register (www.clinicaltrials.gov; identifier NCT00100269) and the Therapeutic Goods Authority (TGA), Australia.

Information on demographics, pregnancy history, prior IVF treatment and baseline sperm parameters were collected for all couples, as outlined in Table 2. Those subjects eligible for enrolment were randomly allocated to the active Menevit antioxidant or a placebo at a ratio of 2 : 1. This uneven allocation was deemed necessary when pre-trial surveys suggested that if participants were offered a 50% chance of receiving active antioxidant treatment, many would self-supplement with 'over the counter' antioxidants. This was deemed less likely with a 2 : 1 active to placebo allocation. The randomisation schedule was computer generated in blocks of six by Bayer Consumer Care Australia, and the appropriately numbered bottles of capsules delivered to the clinical site without any clinical participant knowing the treatment sequence. Patients were allocated the next numerical treatment package (one to 60) as they became eligible for enrolment. The active Menevit and placebo were identical in appearance and taste. Male participants were asked to take one capsule per day after food, starting three months prior to their partners IVF oocyte retrieval. Subject compliance and side-effects were assessed by a questionnaire completed by the male partner on the day of oocyte retrieval.

The IVF procedures consisted of a typical long down regulation protocol with GnRH agonist (nafarilin acetate or leuprolide acetate) commencing in the mid-luteal phase of the preceding cycle. At day two of the stimulation cycle, women were commenced on 150–300 IU of rFSH (Puregon, Organon, Sydney, Australia or Gonal-F, Serono) depending on their age and previous IVF response. Ovarian response was tracked by pelvic ultrasound and serum estradiol, with 5000 IU hCG (Pregnyl, Organon) being administered when at least two follicles were ≥ 18 mm in size with an adequate

Table 2 Participant baseline demographic and fertility characteristics

	Active Menevit	Placebo	<i>P</i>
Female age (years)	34.6 ± 3.4	33.6 ± 3.9	NS
Duration infertility (years)	4.2 ± 2.7	3.4 ± 2.1	NS
Gravidity	0.77 ± 0.9	0.55 ± 0.8	NS
Aetiology of infertility			
Male	45%	50%	NS
Combined	55%	50%	
Prior IVF treatment (%)	52.5%	55%	NS
Number prior IVF cycles	1.8 ± 1.9	1.5 ± 1.9	NS
Oocytes in prior IVF cycle	10.5 ± 4.3	9.9 ± 2.6	NS
Fertilisation rate prior IVF cycle (%)	56.9%	57.5%	NS
Prior IVF embryo quality (%)			
Grade 1 (excellent)	14.7%	15.8%	NS
Grade 2 (good)	33.7%	47.3%	
Grade 3/4 (poor)	51.6%	36.8%	
Male age (years)	37.1 ± 5.1	35.5 ± 4.3	NS
Sperm concentration (NR ≥ 20 × 10 ⁶ /mL)	26.1 ± 26.4	26.7 ± 27.5	NS
Sperm motility (NR ≥ 50%)	32.1 ± 15.9	36.4 ± 13.8	NS
Sperm normal morphology (NR ≥ 20%)	5.1 ± 4.1	6.8 ± 4.4	NS
Sperm vitality – HOST (NR ≥ 60%)	55.5 ± 12.6	56.0 ± 18.7	NS
DNA fragmentation (NR < 25%)	37.9 ± 11.9	40.3 ± 15.3	NS

Values are mean ± SD.

IVF, *in vitro* fertilisation; NR, normal range for fertile men.

estradiol response. Transvaginal oocyte retrieval was conducted under sedation 36 h after hCG administration, followed by standard ICSI fertilisation procedures. Cleavage stage embryos were graded according to traditional morphological criteria (blastomere shape, number and percentage fragmentation) and returned to the uterus on day two or three post-oocyte collection under ultrasound guidance. Only a minority of patients in this trial used elective blastocyst culture and transfer (30% Menevit, 25% placebo) as extended culture was not routinely practiced in our unit. All patients had luteal support using a combination of Crinone 8% vaginal progesterone (Serono) and a single 500 IU injection of hCG on day six post-oocyte retrieval. Serum pregnancy tests were performed 16 days after oocyte retrieval in the absence of a menstrual period. First trimester pregnancy scans were conducted at 8 weeks gestation using a 7.5-MHz transvaginal scanner (Toshiba, Tokyo, Japan). All pregnancies were then followed out to the end of the first trimester (13 weeks gestation).

The primary outcome for this trial was the number of good quality embryos generated per IVF cycle. This was chosen as the primary outcome rather than pregnancy rates, as preliminary power calculations using pregnancy rates revealed a sample size too large for a single site trial. Pilot observations within our unit suggested that men with high levels of oxidative DNA fragmentation produced only two good quality embryos per IVF-ICSI cycle. We therefore powered our study to detect a minimum increase of one good quality embryo (from two to three good quality embryos) per IVF cycle initiated. This power analysis revealed that 60 IVF-ICSI cycles would detect a clinically significant difference between groups, assuming a power of 80%; two-sided testing

at the 5% significance level and a 10% IVF dropout rate. Pregnancy outcome, fertilisation rates and male side-effect profiles were secondary outcomes.

Data were analysed using commercial software (SPSS 11.5.1; SPSS Inc., Chicago, IL, USA). Baseline demographic and fertility-related variables between groups were analysed using unpaired *t*-test for continuous variables and χ^2 for categorical variables. Differences in embryo quality and pregnancy outcome were analysed by χ^2 analysis. A *P*-value ≤ 0.05 was considered statistically significant. Data were analysed on an 'intention-to-treat' basis, irrespective of male medication compliance.

Results

A total of 82 men were screened for entry into the trial, with 22 being excluded because of low levels of sperm DNA damage or no evidence of oxidative stress. Six study participants withdrew from IVF treatment during the study (two from the active arm, four from the placebo arm). One participant in the active medication arm did not reach an embryo transfer as no embryos were available because of immediate oocyte lysis at time of ICSI. Another active arm participant was unable to have a fresh embryo transfer because of a severe ovarian hyperstimulation. No participant withdrew from the study because of spontaneous conception prior to trial exit. However, two participants on the active Menevit medication did conceive spontaneously within one month of exiting the trial (data not included in study outcome analysis).

The baseline characteristics of trial participants are recorded in Table 2. There were no significant differences

Table 3 *In vitro* fertilisation cycle outcomes by study group

	Active Menevit	Placebo	<i>P</i>
Number of oocytes collected	11.4 ± 4.4	9.6 ± 3.9	0.15
Metaphase II oocytes injected	9.3 ± 3.8	7.9 ± 3.2	0.19
Fertilisation rate (%)	68.8%	63.0%	NS
Embryo quality			
Grade 1 (excellent)	11.6%	13.7%	
Grade 2 (good)	44.2%	37.6%	
Grade 3/4 (poor)	44.2%	48.7%	
Embryos transferred	1.39 ± 0.6	1.56 ± 0.5	0.33
Embryos cryo-preserved	1.71 ± 0.5	1.40 ± 0.5	0.32

Values are mean ± SD.

Table 4 Pregnancy outcomes by study group

	Active Menevit	Placebo	<i>P</i>
Embryo transfer procedures	36	16	
Total number of embryos transferred	52	25	
Biochemical pregnancy	3	–	
Clinical miscarriage	2	2	
Ectopic pregnancy	1	–	
Viable singleton	13	4	
Viable singleton/non-viable twin	1	–	
Viable twin	3	–	
Pregnancy rate (positive βhCG)	23/36 (63.9%)	6/16 (37.5%)	0.077
Implantation rate†	24/52 (46.2%)	6/25 (24%)	0.062
Viable pregnancy rate‡	20/52 (38.5%)	4/25 (16%)	0.046

†Implantation rate calculated as the percentage of transferred embryos resulting in a clinical pregnancy (gestational sac) on first trimester scan.

‡Viable pregnancy rate calculated as the percentage of transferred embryos resulting in a viable pregnancy at 13 weeks gestation.

between the active and the placebo group in terms of important baseline prognostic characteristics such as maternal/paternal age, past reproductive history, aetiology of infertility and entry sperm parameters. Furthermore, the prior IVF experiences between each group were not significantly different (Table 2). This would suggest that randomisation had been successful in equally distributing the important confounding variables between the two groups.

While no differences in the primary study outcome of embryo quality were observed between the two trial arms (Table 3), the pregnancy outcomes were significantly better in the antioxidant treatment arm compared to the placebo (Table 4). The Menevit implantation rate was almost double that of the placebo (46.2 vs 24%, $P = 0.06$), with the differences in viable fetal hearts at 13 weeks gestation (38.5 vs 16%) being statistically significant ($P = 0.046$).

A total of 55 men completed the side-effects questionnaire (92% return rate). Compliance with taking the medication was excellent with 96% of participants missing less than one capsule per week. None of the men on the placebo noted any side-effects. In the Menevit group, three of the 37 men (8%) noted mild side-effects. Two reported mild gastro-esophageal reflux and the third constipation. No participant felt that the

side-effects were significant enough to consider withdrawing from the trial.

Discussion

To the best of our knowledge this study is the first RCT showing that administration of an antioxidant can improve pregnancy rates during IVF-ICSI treatment. Previous RCTs have shown a reduction in free radical-mediated damage to sperm^{12,25} and improvements in sperm DNA integrity¹⁴ but had not monitored pregnancy outcomes. A recent non-randomised study²⁶ suggested that a combination of vitamins C and E could boost pregnancy rates during IVF-ICSI treatment in men with high sperm DNA damage. However, this trial was weakened by the use of patients' prior IVF pregnancy outcome as a historical control. This is inherently misleading as pregnancy rates can only improve in such a setting (no viable pregnancies at entry, hence the need for further IVF treatment), irrespective of any biological effect of the trial treatment.

Our study was unable to detect any significant effect of antioxidant medication on cleavage stage embryo quality that

could explain the observed improvements in pregnancy rates. This is consistent with the findings of Greco *et al.*²⁶ who also found that a combination of vitamins C and E had no effect on cleavage stage embryo quality, despite a reduction in sperm DNA fragmentation and a boost in pregnancy rates. In addition, previous work examining the link between seminal ROS levels and embryo quality found no relationship between ROS and day two embryo quality following IVF-ICSI, yet did note a significant negative correlation between high levels of ROS and poor blastocyst formation rates.²⁷ These results suggest that oxidative stress-related DNA fragmentation does not manifest itself as changes in embryo quality until the embryos are cultured beyond day three, when the paternal genome begins to be transcribed. Blastocyst culture is possibly a better marker of sperm DNA integrity than cleavage stage assessment. Unfortunately, as only 28% of participants underwent blastocyst culture, definitive comparison of blastocyst development rates was impossible because of the small sample size.

Antioxidant treatment did not produce a significant improvement in IVF-ICSI fertilisation rates. This observation is consistent with the reported literature.^{27,28} Zorn *et al.*²⁷ reported a negative correlation between seminal ROS levels and routine IVF fertilisation rates but not when performing ICSI. Normally, when free radicals damage the sperm membrane, the damaged sperms exhibit impaired fusion with the oocyte membrane, thereby preventing fertilisation. However, ICSI bypasses this normal fertilisation process and as a result there is no impediment to creation of an embryo despite ROS-related sperm damage. Bypassing this 'fertilisation block' using ICSI is potentially of major clinical concern. Fragmented sperm DNA has been linked with recurrent miscarriage,²⁹ childhood cancer and possibly even autosomal dominant disorders such as achondroplasia.³⁰ Since antioxidants such as vitamins C and E have been shown to reduce sperm DNA fragmentation,¹⁴ and lycopene, zinc and selenium are known to protect somatic cells from oxidative-related DNA damage,^{31–33} it may be prudent to consider using antioxidants in all men undergoing IVF-ICSI treatment. Hopefully, this treatment will lead to not only improved IVF-ICSI pregnancy rates but may also reduce serious health problems in the resulting children. Of course, very large studies will be required to prove the effectiveness of antioxidants in preventing rare disorders such as childhood cancers and autosomal dominant conditions.

A recent study reported that 31% of men in an infertile relationship take alternative therapies, with the number one alternative being antioxidant containing preparations such as vitamin C, vitamin E, zinc and selenium.³⁴ Patients embrace 'natural' therapies such as antioxidants as they are perceived as safe and their easy accessibility 'over the counter' gives patients some control over their own treatment. During this study many men commented that they welcomed the opportunity to actively influence their own IVF treatment outcome.

The present study is the first randomised double-blind placebo-controlled study to show that an antioxidant has the ability to improve pregnancy rates during IVF-ICSI treatment. While the underlying mechanism is presently unclear, previous work suggest that improvements in sperm DNA damage are

likely to be responsible.^{14,31,32} Future studies examining changes in sperm DNA fragmentation and blastocyst development resulting from antioxidant treatment will hopefully shed light on the mechanisms behind the observed improvements in pregnancy rates.

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References

- 1 McLachlan RI, de Kretser DM. Male infertility: The case for continued research. *MJA* 2001; 174: 116–117.
- 2 Waters AM, Dean JH, Sullivan EA. *Assisted Reproduction Technology in Australia and New Zealand 2003*. Sydney, Australia: AIHW National Perinatal Statistics Unit, 2006.
- 3 de Lamirande E, Gagnon C. Human sperm hyper-activation and capacitation as parts of an oxidative process. *Free Radic Biol Med* 1993; 14: 157–166.
- 4 Zini A, de Lamirande E, Gagnon C. Low levels of nitric oxide promotes human sperm capacitation in vitro. *J Androl* 1995; 16: 424–431.
- 5 Agarwal A, Prabakaran S, Allamaneni S. What an andrologist / urologist should know about free radicals and why. *Urology* 2006; 67: 2–8.
- 6 Garrido N, Meseguer M, Simon C, Pellicer A, Re J. Pro-oxidative and anti-oxidative imbalance in human semen and its relationship with male fertility. *Asian J Androl* 2004; 6: 59–65.
- 7 Aitken J, Krausz C, Buckingham D. Relationships between biochemical markers for residual sperm cytoplasm, reactive oxygen species generation, and the presence of leukocytes and precursor germ cells in human sperm suspensions. *Mol Reprod Dev* 1997; 47: 468–482.
- 8 Aziz N, Saleh RA, Sharma RK *et al.* Novel association between sperm reactive oxygen species production, sperm morphological defects, and the sperm deformity index. *Fert Steril* 2004; 81: 349–354.
- 9 Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W. Total antioxidant capacity of seminal plasma is different in fertile and infertile men. *Fert Steril* 1995; 64: 868–870.
- 10 Alkan I, Simsek F, Haklar G *et al.* Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: Relationship to seminal plasma antioxidants. *J Urol* 1997; 157: 140–143.
- 11 Agarwal A. Role of antioxidants in treatment of male infertility: An overview of the literature. *Reprod Biomed Online* 2004; 8: 616–627.
- 12 Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: Protective role of vitamin E. *J Androl* 1996; 17: 530–537.
- 13 Rolf C, Cooper TG, Yeung CH, Nieschlag E. Antioxidant treatment of patients with asthenozoospermia or moderate

- oligoasthenozoospermia with high-dose vitamin C and vitamin E: A randomized, placebo-controlled, double-blind study. *Hum Reprod* 1999; 14: 1028–1033.
- 14 Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl* 2005; 26: 349–353.
 - 15 Opara EC, Rockway SW. Antioxidants and micronutrients. *Dis Mon* 2006; 52: 151–163.
 - 16 Heber D, Lu QY. Overview of mechanism of action of lycopene. *Exp Biol Med* 2002; 227: 920–923.
 - 17 Prasad K, Laxdal VA, Yu M, Raney BL. Antioxidant activity of allicin, an active principal in garlic. *Mol Cell Biochem* 1995; 148: 183–189.
 - 18 Chung LY. The antioxidant properties of garlic compounds: Allyl cysteine, alliin, allicin and allyl disulphide. *J Med Food* 2006; 9: 205–213.
 - 19 Hodge G, Hodge S, Han P. *Allium sativum* (garlic) suppresses leukocyte inflammatory cytokine production in vitro: Potential therapeutic use in the treatment of inflammatory bowel disease. *Cytometry* 2002; 48: 209–215.
 - 20 Pfeifer H, Conrad M, Roethlein D *et al.* Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. *FASEB J* 2001; 15: 1236–1238.
 - 21 Kvist U, Bjorndahl L, Kjellberg S. Sperm nuclear zinc, chromatin stability and male fertility. *Scanning Microscopy* 1987; 1: 1241–1247.
 - 22 Huang RF, Ho YH, Lin HL, Wei JS, Liu TZ. Folate deficiency induces a cell cycle-specific apoptosis in HepG2 cells. *J Nutr* 1999; 129: 25–31.
 - 23 World Health Organization. *Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucous Interaction*, 4th edn. New York: Cambridge University press, 1999.
 - 24 Lopes S, Sun JG, Jurisicova A, Meriano J, Casper RF. Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. *Fertil Steril* 1998; 69: 528–532.
 - 25 Keskes-Ammar L, Feki-Chakroun N, Rebaj T *et al.* Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 2003; 49: 83–94.
 - 26 Greco E, Romano S, Iacobelli M *et al.* ICSI in cases of sperm DNA damage: Beneficial effect of oral anti-oxidant treatment. *Hum Reprod* 2005; 20: 2590–2594.
 - 27 Zorn B, Vidmar G, Meden-Vrtovec H. Seminal reactive oxygen species as predictors of fertilization, embryo quality and pregnancy rates after conventional in vitro fertilization and intracytoplasmic sperm injection. *Int J Androl* 2003; 26: 279–285.
 - 28 Twigg JP, Irvine DS, Aitken RJ. Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. *Hum Reprod* 1998; 13: 1864–1871.
 - 29 Carrell DT, Liu L, Peterson CM *et al.* Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. *Arch Androl* 2003; 49: 49–55.
 - 30 Aitken RJ, Baker MA, Sawyer D. Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. *Reprod Biomed Online* 2003; 7: 65–70.
 - 31 Rehman A, Bourne LC, Halliwell B, Rice-Evans CA. Tomato consumption modulates oxidative DNA damage in humans. *Biochem Biophys Res Commun* 1999; 262: 828–831.
 - 32 Porrini M, Riso P. Lymphocyte lycopene concentration and DNA protection from oxidative damage is increased in women after a short period of tomato consumption. *J Nutr* 2000; 130: 189–192.
 - 33 Klotz LO, Kroncke KD, Buchczyk DP, Sies H. Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. *J Nutr* 2003; 133: 1448–1451.
 - 34 Zini A, Fisher MA, Nam RK, Jarvi K. Use of alternative and hormonal therapies in male infertility. *Urology* 2004; 63: 141–143.