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Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis

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BACKGROUND: We have recently shown that patients with prostato-vesiculo-epididymitis (PVE) have a greater reactive oxygen species (ROS) overproduction than patients with prostatitis or prostato-vesiculitis. Since this biochemical stress persists even after treatment with antimicrobials, it may relate to an imbalance between pro- and anti-oxidant factors at the epididymal level. METHODS: To evaluate the effects of antioxidant treatment of patients with PVE, whether in the presence or absence of pro-oxidant factors, abacterial PVE infertile patients with normal ($<1 \times 10^6$/ml, group A, $n = 34$) or abnormal ($>1 \times 10^6$/ml, group B, $n = 20$) seminal white blood cell (WBC) concentrations received carnitines (L-carnitine 1 g and acetyl-carnitine 0.5 g twice/day) for 3 months followed by a wash-out period of 3 months. Semen parameters, ROS production and pregnancy outcome were evaluated before, during and following carnitine treatment. RESULTS: Carnitines increased sperm forward motility and viability in group A patients. This was associated with a significant reduction in ROS production which persisted during wash-out. Carnitines increased only the percentage of viable spermatozoa in group B patients. Within 3 months after the discontinuation of carnitines, the rate of spontaneous pregnancy in group A patients was significantly higher than that of group B patients, being 11.7% (4/34) compared with 0%. CONCLUSION: These results indicate that carnitines are only an effective treatment in patients with abacterial PVE and elevated ROS production when seminal WBC concentration is normal.

Key words: carnitines/leucocytospermia/prostato-vesiculo-epididymitis/reactive oxygen species/sperm parameters

Introduction

Recently, we have reported a stronger association between sexual gland post-inflammation damage (shown by an abnormal ultrasound appearance), increased white blood cell (WBC) concentration and reactive oxygen species (ROS) production in patients with prostato-vesiculo-epididymitis (PVE) compared with patients with prostatitis or prostato-vesiculitis (Vicari, 1999). Furthermore, following antimicrobial treatment, patients with PVE had the lowest bacteriological cure rate (52%) of all, even after three antibiotic courses, as well as persistent infertility, dyspermia and ROS over-production of sperm origin (Vicari, 2000). In these patients, the poor therapeutical outcome could be secondary to a hostile epididymal microenvironment due to the residual presence of pro-oxidant factors (infection, presence of some cytokines, etc.) (Omu *et al*., 1998) and/or inadequate antioxidant activity (epididymal secretory dysfunction) (Ochsendorf, 1999) even following antimicrobial treatment. The antioxidant properties of some pharmacologically available natural molecules (carnitine, vitamins, etc.) could become a promising strategy in the course of anti-infectious treatment of these patients (Ochsendorf, 1999), in case a re-equilibrium between leukocytes and/or sperm ROS production and anti-oxidant scavenger activity needs to be reached in the seminal plasma (Ford and Whittington, 1998; Geva *et al*., 1998; Lenzi *et al*., 1998; Tarin *et al*., 1998). Therefore, patients with PVE may represent a valid clinical model for the rational use of antioxidant scavengers and for testing their effectiveness. Indeed, the reported controversial effects of antioxidant compounds on sperm quality (Sikka *et al*., 1995) mainly relate to the fact that this treatment was given to unselected male patients.

Some antioxidants (vitamins E, A and C, glutathione) are able to chain-break ROS-induced lipid peroxidation within the sperm membrane or in the seminal plasma (Sikka, 1995); others (i.e. carnitine/acetyl-carnitine complex) seem to exert a repairing effect through the removal of elevated intracellular toxic acetyl-coenzyme A (acetyl-CoA) and/or the replacement of fatty acid in membrane phospholipids (Arduini, 1992). Furthermore, high concentrations of carnitine, a 3 hydroxy-4 trimethylaminobutyric acid, are present in both seminal plasma and spermatozoa, where carnitine is efficient in the transport of fatty acids through mitochondrial membranes and in the intracellular storage of acetate moieties derived from acetyl-CoA. Acetyl-carnitine, generated by the carnitine acetyltransferase reaction, is a useful compound in the energetic-metabolic

cellular economy through the transport of acetyl-groups into mitochondria, where acetyl-CoA serves as fuel for the Krebs cycle to supply ATP for sperm motility. The concentration of this biochemical complex increases continuously during the epididymal transit, that is when sperm motility and fertilizing ability develop, thus suggesting that the carnitine-acetylcarnitine complex may play an important role in these processes (Brooks, 1980). Since the important antioxidant role of the epididymal microenvironment has been pointed out (Potts *et al*., 1999), we thought that a combined treatment with carnitines (carnitine and acetyl-carnitine complex) might be rational in conditions of acetyl-CoA accumulation. Indeed, carnitine and acetyl-carnitine act as the first and second scavenger agent respectively to remove acetyl-CoA from the cell, and possibly replace unsaturated fatty acids in the plasma membrane phospholipids (Arduini, 1992). In addition, a combined treatment reduces the dosage of each antioxidant and reproduces more closely the biochemical conditions present in the epididymal milieu. For these reasons, we administered carnitine plus acetyl-carnitine to 54 asymptomatic infertile patients with ultrasound evidence of PVE. The patients became abacterial after antimicrobial treatment but proved to have a persistent seminal ROS over-production and ultrasound evidence of PVE. On the basis of seminal WBC concentration, they were divided into two groups: group A with normal WBC ($<1\times10^6$/ml) and group B with elevated WBC ($>1\times10^6$/ml) concentration. Sperm parameters, ROS production and spontaneous pregnancy rates were evaluated before, during and following treatment with carnitines.

Materials and methods

Patient selection

Fifty-four patients (median age: 32 years, range: 24–42) affected by primary infertility (median duration: 6.8 years, range 3–13) and male accessory gland inflammation (MAGI), diagnosed according to World Health Organization (WHO) clinical and laboratory criteria (World Health Organization, 1993), were enrolled in the study. No female infertility factors were apparently present, since all female partners (median age: 31 years, range: 24–37) were ovulating regularly, as indicated by biphasic basal body temperature and a follicular ultrasound scan, luteal phase progesterone levels, or endometrial biopsy. Tubal patency was assessed by hysterosalpingogram or laparoscopy in all women. A complete reproductive and sexual history was obtained and a physical examination was performed on each male patient. Sixteen out of 54 couples (10 before and six after antibiotic treatment) had failed a previous IVF/embryo transfer programme, because of failure of fertilization (fertilization rate, FR = 0%) in all cases.

Inclusion criteria

All infertile patients were affected by chronic abacterial PVE, suspected on the basis of the following eligibility criteria: (i) oligo- (sperm concentration $<20\times10^6$/ml), astheno- (<50% spermatozoa with forward progression, a and b categories) or terato- (<30% spermatozoa with normal oval form) -zoospermia; (ii) clinical signs (at the physical examination) and ultrasound findings considered indicative of chronic PVE, as previously described (Vicari, 1999, 2000); (iii) achievement of a bacteriological cure ($<1\times10^3$ colony-forming units, CFU/ml), following antimicrobial treatment with ofloxacin (200 mg orally every 12 h; Flobacin®, SigmaTau, Italy) or doxycycline (100 mg orally once daily; Bassado®, Poli, Italy) for 14 days/month over a 3 month period, in patients with one or two consecutive cultures with significant bacteriospermia ($\geq10^5$ CFU/ml), or eradication of *Chlamydia trachomatis* or *Ureaplasma urealyticum* from cultures of urethral swabs obtained after prostatic massage, following the same antimicrobial treatment; and (iv) seminal ROS over-production after antimicrobial treatment.

Exclusion criteria

(i) PVE patients with a significant microbial reinfection (with growth of $\geq10^5$ *Streptococcus faecalis* or *Staphylococcus aureus* species) resistant (minimal inhibitory concentration, MIC, 8–16 mg/l) to both drugs (ofloxacin or doxycyclin). The cut off value of $\geq10^5$ CFU/ml, much higher than the value mentioned in the WHO semen manual (WHO, 1992) was chosen because of its recently reported strong association with ultrasound abnormalities (Vicari, 1999); (ii) azoospermia, severe oligozoospermia ($<5\times10^6$/ml), terato-zoospermia (normal forms <14%) according to the WHO criteria (World Health Organization, 1992), and/or necrozoospermia (sperm viability <10%); (iii) elevated (>10 mIU/ml) serum FSH concentrations; (iv) history or presence of primary testicular disease (cryptorchidism, orchitis, varicocele) or testicular volume ≤12 ml; (v) smoking habit, alcohol consumption, occupational chemical exposure; history of major renal and hepatic disorders and myopathy; and (vi) treatment with other drugs within the 3 months before enrolment in this study.

Study design and treatments

Before initiating antioxidative treatment, patients were subdivided into two groups according to their seminal WBC concentration: group A (n = 34) had normal seminal WBC ($<1\times10^6$/ml); group B (n = 20) had elevated seminal WBC ($>1\times10^6$/ml). The patients of both groups received carnitine (1 g, Carnitene®, Sigma-Tau, Pomezia-Rome, Italy) and acetyl-carnitine (500 mg, Nicetile®, Sigma-Tau) orally every 12 h for 3 months, followed by a wash-out period of 3 months.

This study was approved by the Committee for Ethics of the Medical Faculty, University of Catania. All patients provided their written informed consent. They completed the entire trial, including treatment and follow-up examinations.

Sampling management

All the patients underwent semen analyses before (T0) and during treatment (T3), and 90 days (range 90–101 days) after completion of therapy (T6). After liquefaction, semen samples were analysed for sperm concentration, total sperm number, forward motility percentage (% grade a + b, after 1 h), sperm morphology (percentages of normal oval forms), sperm viability (percentages of viable spermatozoa) and seminal WBC concentration according to the WHO guidelines (World Health Organization, 1992). In particular, conventional immunocytochemical staining (World Health Organization, 1992) was used to assess seminal WBC concentration, according to previously published methods (Vicari, 1999). All semen analyses were performed by the same investigator in a blinded fashion, to minimize methodological errors. Spermatozoa were separated by means of a two-step 45/90% discontinuous Percoll gradient; basal and formyl-methionyl-leucyl-phenylalanine (Sigma Chemicals Co., St Louis, MO, USA) (fMLP)-stimulated ROS measurements were then carried out on 400 µl aliquots of cell suspension both from the 90% Percoll layer and the 45/90% Percoll interface (= 45% Percoll fraction), as previously reported (Vicari, 1999). ROS production was measured in aliquots containing a maximum of 2.5×10^6 spermatozoa/ml to reduce the

Table I. Sperm parameters in patients with abacterial prostato-vesiculo-epididymitis and normal ($<1\times10^6$/ml; group A) or abnormal ($>1\times10^6$/ml; group B) seminal white blood cell (WBC) concentrations

Sperm parameters	Group A (n = 34)	Group B (n = 20)
Concentration (x10^6/ml)		
T0	14.0 (10–58)	12.5 (9–46)
T3	18.5 (13–58)	17.0 (11–56)
T6	16.0 (11–52)	15.5 (11–51)
Total sperm number (x10^6/ejaculate)		
T0	32.0 (28–118)	28.3 (19–103)
T3	44.0 (28–157)	31.5 (21–118)
T6	37.0 (27–131)	26.5 (20–101)
Forward motility (%)		
T0	14 (10–20)[a]	10.5 (8.5–15)
T3	28.0 (22–35)[a]*	16.0 (11.5–22)
T6	20.0 (11–25)	15.0 (10.0–20)
Normal forms (%)		
tT0	20 (15–35)	17.5 (15–31)
T3	26 (18–40)	21.5 (15–34)
T6	22 (17–38)	20.0 (17–32)
Viability (%)		
T0	29.5 (25–32)[a]	27.5 (25–40)[a]
T3	42.0 (32–56)[a]*	33.0 (28–46)[a]
T6	35.0 (28–38)	31.0 (26–45)
WBC (x10^6/ml)		
T0	0.8 (0.7–0.9)*	1.6 (1.1–1.9)[a,b]
T3	0.75 (0.7–0.9)*	1.2 (1.0–1.4)[a]
T6	0.8 (0.7–0.9)*	1.2 (1.0–1.4)[b]

Values are expressed as median values and the 10th and 90th percentiles are in parentheses.
n = number of patients treated; T0 = pre-treatment; T3 = 3 month treatment with carnitines; T6 = 3 months after washout period
[a,b]Values with the same superscripts are statistically different within the same group ($P < 0.05$, Duncan's test).
*$P < 0.01$ for comparison with group B.

number of WBC in each aliquot and consequently the number of 'overflow' samples. The counts obtained in aliquots containing $<2.5\times10^6$ spermatozoa/ml were adjusted to this number.

Safety assessment

Safety assessment included medical history, physical examinations, haematological and serum chemistry profiles at all visits, and the monitoring of drug-related adverse events by means of indirect questioning through patients' diaries.

Statistical analysis

In every phase of the trial, sperm results are the mean of two consecutive semen specimens, collected 3–5 days apart. Throughout the text, results are shown as median and the 10th and 90th percentiles in parentheses. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was applied to evaluate the effects of treatment within each group. The effects of carnitine treatment between the two groups were evaluated by means of the Mann–Whitney U-test for unpaired data. Fisher's exact test was used to compare the rates of spontaneous pregnancy (and pregnancy rate per cycle) between the groups. P value < 0.05 was considered statistically significant.

Results

Sperm output

The sperm parameters observed during the trial are reported in Table I. Sperm concentration, total sperm number and percentage of normal forms did not show any significant change between the two groups. Forward motility increased for group A patients after treatment with carnitines, whereas it did not change in group B. Viability improved significantly in patients of both groups after treatment with carnitines. Group B patients showed a significant reduction in seminal WBC after treatment with carnitines and this diminution persisted at the 3 month post-treatment follow-up, although these median values remained higher than those found in group A patients (Table I).

ROS production

Basal and fMLP-stimulated ROS production are summarized in Table II. The treatment with carnitines significantly reduced basal and fMLP-stimulated ROS productions in group A patients. This reduction was still noticeable after 3 months of wash-out. In contrast, the treatment with carnitines did not have any significant effect on group B patients, whose basal and fMLP-stimulated ROS productions were significantly higher that those observed in group A patients.

Spontaneous pregnancies

Four patients from group A (11.7%) impregnated their partners spontaneously within 3 months from the discontinuation of carnitine treatment (3.1% pregnancy rate per cycle). None of the patients from group B achieved a spontaneous pregnancy.

Safety assessment

The carnitines administered throughout the trial were generally well tolerated. No laboratory abnormalities were observed.

Discussion

Since ROS overproduction has been associated with defective sperm function (Aitken *et al*., 1989) and the incidence of spontaneous pregnancy has been shown to be negatively correlated with the generation of ROS (Aitken *et al*., 1991), many infertile patients have been treated with antioxidant compounds (vitamins, gluthatione, ubiquinol and carnitine alone) (Campaniello *et al*., 1989; Moncada *et al*., 1992; Lenzi *et al*., 1993; Costa *et al*., 1994; Sikka *et al*., 1995; Vitali *et al*., 1995). However, the effect of this treatment on sperm quality is still an open question, mostly because of an indiscriminate selection of the patient cohort who antioxidants are administered to (Ford and Whittington, 1998; Geva *et al*., 1998; Lenzi *et al*., 1998; Tarin *et al*., 1998; Comhaire *et al*., 1999).

Previous studies have demonstrated an oxidative sperm stress secondary to an unbalanced pro-oxidant/antioxidant ratio in patients with MAGI. They have shown a direct relationship between seminal WBC concentrations and the seminal concentration of some cytokines, and an inverse relationship between ROS production and the seminal concentrations of gamma-glutamyltransferase (γ-GT) and α-glucosidase, which are known biochemical markers of epididymal origin (Depuydt *et al*., 1996). Furthermore, antimicrobial treatment produced only a limited antioxidant effect on these patients, since it improved some sperm parameters associated with ameliorated

Table II. Changes in basal and formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated reactive oxygen species (ROS) in 45% and 90% Percoll fractions in patients with abacterial prostato-vesiculo-epididymitis (PVE) and normal ($<1\times10^6$/ml; group A) or abnormal ($>1\times10^6$/ml; group B) seminal white blood cell concentrations. Basal and fMLP-stimulated ROS productions in normal fertile men are shown for comparison

	45% Percoll fraction		90% Percoll fraction	
	baseline ROS	fMLP-ROS	baseline ROS	fmlp-ROS
Group A (n = 34)				
Pre-treatment (T0)	74.4 (48.5–247.2)[a,b]	81.4 (56.9–262.2)[a,b]	42.6 (21.8–68.1)[a,b]	5.4 (22..8–75.2)[a,b]
Carnitines (T3)	40.8 (28.3–98.7)[a,]	47.1 (32.3- 67.5)[a,]	18.5 (12.3–25.3)[a]	20.3 (13.7–29.6)[a]
Wash-out (T6)	55.2 (37.5–190.3)[b]	63.2 (43.7–198.7)[b]	31.2 (18.7–42.5)[b]	436.0 (20.8–46.7)[b]
Group B (n = 20)				
Pre-treatment (T0)	99.7 (79.2–511.5)°	155.8 (123.2–564.0)*	61.1 (30.2–79.5)	58.0 (32.4–68.8)
Carnitines (T3)	81.2 (61.7–406.5)*	129.3 (87.1–860.7)*	48.8 (26.2–66.8)*	42.8 (26.7–51.0)*
Wash-out (T6)	84.9 (68.6–410)	141.2 (87.5–508.6)*	50.0 (22.6–55.3)	55.2 (29.7–62.3)
Fertile (n = 15)	38.0 (14–40.2)	58.7 (23.9–85.1)	10.7 (8.3–18.8)	12.2 (9.5–21.1)

Values (c.p.m.×1000) are median and 10th and 90th percentiles are in parentheses.
n = number of patients.
[a,b]Values with the same superscripts are statistically different within the same group ($P < 0.05$, Duncan's multiple test).
°$P < 0.05$ for comparison with group A; *$P < 0.01$ for comparison with group A.

antioxidant proprieties of the seminal plasma through increased interleukin (IL)-4 levels, whilst the levels of IL-2 and IL-8 remained low (Omu *et al*., 1998). In other words, this limited antioxidant effect may be explained by an increased seminal IL-4 concentration, which has marked inhibitory effects on the expression and release of the pro-inflammatory cytokines produced by T helper 1-type lymphocyte (IL-2), monocytes and macrophages (IL-8). Moreover, peroxidative damage could be caused by the growth of bacterial mycotoxins, whose effects have been counteracted by in-vitro addition of antioxidants (co-enzyme Q-10 plus carnitine) (Atroshi *et al*., 1998). Recently, we have shown that, in comparison with patients affected by prostatitis or prostato-vesiculitis, patients with PVE have the highest leukocytic/juxta-spermatozoa ROS over-production (Vicari, 1999) associated with the lowest antimicrobial efficacy, in terms of bacteriological cure rate, pregnancy rate, sperm outcome and a persistent ROS over-production (Vicari, 2000). This suggests that PVE patients may represent a well-defined clinical model of oxidative stress for evaluating the antioxidant effects of carnitines.

Following the removal of the microbial noxae, 20 out of 54 patients (37%) still exhibited an increased concentration of seminal WBC (group B). Activated leukocytes can enhance ROS production by mature and immature spermatozoa (Ochsendorf, 1999; Piquet-Pellorce *et al*., 2000) thus having detrimental effects on sperm function. In contrast, the other PVE patients studied showed a normalization of seminal WBC concentration. Nevertheless, they had a persistent oxidative stress of sperm origin (basal but not fMLP-stimulated ROS production). This may relate to an absolute or relative increase in pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), IL-1β or IL-8, etc., and/or their soluble receptors, not necessarily associated with an increase in seminal WBC concentration. It has been shown that male germ cells are able to produce TNF-α (De *et al*., 1993) and there is a mounting body of evidence that demonstrates the importance of cytokines and chemokines in testicular paracrine regulation (Dousset and Hussenet, 1997; Hales *et al*., 1999).

Treatment with carnitines improved sperm forward motility and viability in PVE patients with normal seminal WBC concentration, besides significantly increasing their otherwise poor (Vicari, 2000) reproductive performance. These effects may be explained by a re-equilibrium of the seminal oxidative balance resulting from an amelioration of the scavanger properties of the epididymal microenvironment. In addition, since exposure to spermiotoxic noxae present at the epididymal levels ('epididymal hostility') (Wilton *et al*., 1988; Ochsendorf, 1999) could be aggravated by the duration of epididymal transit, which seems to be slower in oligozoospermic patients (Johnson and Varver, 1988), it cannot be excluded that the improvement of some sperm parameters may also be due to a more effective energy supply to spermatozoa. This, in turn, reduces transit time and/or protects the sperm pool of the 'epididymal reserve'. The associated reduction in ROS over-production supports the hypothesis that these alterations may be caused by plasma membrane peroxidative damage and/or abnormal or unbalanced levels of pro-inflammatory cytokines. Accordingly, TNF-α and interferon-γ have been shown to be capable of causing astheno-necrozoospermia in the absence of infection when co-incubated with normal spermatozoa (Depuydt *et al*., 1996). Moreover, since several types of bacteria are capable of altering the host cell cytokine synthesis by degrading pro-inflammatory cytokines and/or using cytokine receptors as portals of entry for cellular invasion (Wilson *et al*., 1998), it can be speculated that the administration of carnitines, as it acts on intraleukocytic metabolism, may use some components of the cytokine network to enhance sperm function. At the same time it may re-establish an equilibrium between pro-inflammatory and anti-inflammatory cytokines, reducing the former and/or increasing the latter. On the other hand, treatment with carnitines was only able to increase the percentage of viable spermatozoa without having any detectable effect on sperm forward motility and ROS over-production. None of the group B patients achieved a spontaneous pregnancy. Since carnitines have been shown to localize in polymorphonuclear leukocytes (Katrib *et al*., 1987), it may be hypothesized that this mechanism contributes to annihilating

their antioxidant and energetic effects at the spermatozoon level.

In conclusion, antioxidant treatment with carnitines is effective in patients with PVE, elevated ROS production and normal seminal WBC concentrations. In this group of patients, these compounds may represent a new rational, non-hormonal, therapeutic frontier. The lower efficacy of carnitine treatment in PVE patients with persistently elevated seminal WBC concentrations has suggested that a different anti-inflammatory/antioxidant strategy should be explored in this subgroup of patients.

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References

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

Aitken, R.J., Irvine, D.S. and Wu, F.C. (1991) Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. *Am. J. Obstet. Gynecol.*, **164**, 542–551.

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

Atroshi, F., Rizzo A., Westermarck, T. *et al*. (1998) Effects of tamoxifen, melatonin, coenzyme Q10, and L-carnitine supplementation on bacterial growth in the presence of mycotoxins. *Pharmacol. Res.*, **38**, 289–295.

Brooks, D.E. (1980) Carnitine in the male reproductive tract and its relation to the metabolism of the epididymis and spermatozoa. In Frenkel, R.A., and McGarry, J.D. (eds.), *Carnitine Biosynthesis, Metabolism and Functions*, Academic Press, New York, pp. 219–235.

Campaniello, E., Petrarolo, N., Meriggiola, M.C. *et al*. (1989) Carnitine administration in asthenospermia (Abstract). *Presented at IV International Congress of Andrology, Firenze.*

Comhaire, F.H., Mahmoud, A.M., Depuydt, C.E. *et al*. (1999) Mechanisms and effects of male genital tract infection on semen quality and fertilizing potential: the andrologist's viewpoint. *Hum. Reprod. Update*, **5**, 393–398.

Costa, M., Canale, D., Filicori, M. *et al*. (1994) L-Carnitine in idiopathic asthenozoospermia: a multicenter study. *Andrologia*, **26**, 155–159.

De, S.K., Chen, H.L., Pace, J.L. *et al*. (1993) Expression of tumour necrosis factor-α in mouse spermatogenic cells. *Endocrinology*, **133**, 389–396.

Depuydt, C.E., Bosmans, E., Zalata, A. *et al*. (1996) The relation between reactive oxygen species and cytokines in andrological patients with or without male accessory gland infection. *J. Androl.*, **17**, 699–707.

Dousset, B. and Hussenet, F. (1997) Cytokines in human semen. A new approach to male infertility. *Press. Med.*, **26**, 24–29.

Ford, W.C.L. and Whittington, K. (1998) Antioxidant treatment for male subfertility: a promise that remains unfulfilled. *Hum. Reprod.*, **13**, 1416–1419.

Geva, E., Lessing, J.B., Lerner-Geva, L. *et al*. (1998) Free radicals, antioxidants and human spermatozoa: clinical implications. *Hum. Reprod.*, **13**, 1415–1424.

Hales, D.B., Diemer, T. and Hales, K.H. (1999) Role of cytokines in testicular function. *Endocrine*, **10**, 201–217.

Johnson, L. and Varver, D.D. (1988) Effect of daily sperm production but not age on transit time of spermatozoa through the human epididymis. *Biol. Reprod.*, **39**, 812–817.

Katrib, K., Adlouni, A.H. and Ferard, G. (1987) Carnitine in human polymorphonuclear leukocytes, mononuclear cells, and platelets. *Am. J. Clin. Nutr.*, **46**, 734–735.

Lenzi, A., Culasso, F., Gandini, L. *et al*. (1993) Placebo-controlled, double-blind, cross-over trial of glutathione therapy in male infertility. *Hum. Reprod.*, **8**, 1657–62.

Lenzi, A., Gandini, L. and Picardo, M. (1998) A rationale for glutatione therapy. *Hum. Reprod.*, **13**, 1419–1421.

Moncada, M.L., Vicari, E., Cimino, C. *et al*. (1992) Effect of acetylcarnitine treatment in oligoasthenospermic patients. *Acta Eur. Fertil.*, **23**, 221–224.

Ochsendorf, F.R. (1999) Infections in the male genital tract and reactive oxygen species. *Hum. Reprod. Update*, **5**, 399–420.

Omu, A.E., al-Othman, S., Mohamad, A.S. *et al*. (1998) Antibiotic therapy for seminal infection. Effect on antioxidant activity and T-helper cytokines. *J. Reprod. Med.*, **43**, 855–864.

Piquet-Pellorce, C., Dorval-Coiffec, I., Pham, M.D. *et al*. (2000) Leukemia inhibitory factor expression and regulation within the testis. *Endocrinology*, **141**, 1136–1141.

Potts, R.J., Jefferies, T.M. and Notarianni, L.J. (1999) Antioxidant capacity of the epididymis. *Hum. Reprod.*, **14**, 2513–2516.

Sikka, S.C., Rajasekaran, M. and Hellstrom, W.J.G. (1995) Role of oxidative stress and antioxidants in male fertility. *J. Androl.*, **16**, 464–468.

Tarin, J.J., Brines, J. and Cano A. (1998) Antioxidants may protect against infertility. *Hum. Reprod.*, **13**, 1415–1416.

Vicari, E. (1999) Seminal leukocyte concentration and related specific radical oxygen species production in different categories of patients with male accessory gland infection. *Hum. Reprod.*, **14**, 2025–2030.

Vicari, E. (2000) Effectiveness and limits of antimicrobial treatment on seminal leukocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. *Hum. Reprod.*, **15**, 2536–2544.

Vitali, G., Parente, R. and Melotti, C. (1995) Carnitine supplementation in human idiopathic asthenospermia: clinical results. *Drugs Exptl Clin. Res.*, **21**, 157–159.

Wilson, M., Seymour R. and Henderson B. (1998) Bacterial perturbation of cytokine networks. *Infect. Immun.*, **66**, 2401–2409.

Wilton, U., Temple-Smith, P.D., Baker, H.W. *et al*. (1988) Human male infertility caused by degeneration and death of sperm in the epididymis. *Fertil. Steril.*, **49**, 1052–1058.

World Health Organization (1992) *Laboratory Manual for the Examination of the Human Semen and Sperm-Cervical Mucus Interaction, 3rd edn* Cambridge University Press, Cambridge, UK.

World Health Organization (1993) *WHO manual for the standardised investigation and diagnosis of the infertile couple*. Cambridge University Press, Cambridge, UK.

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