



Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: A double-blind placebo-controlled study

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Summary

Since sperm require high energy levels to perform their specialised function, it is vital that essential nutrients are available for spermatozoa when they develop, capacitate and acquire motility. However, they are vulnerable to a lack of energy and excess amounts of reactive oxygen species, which can impair sperm function, lead to immotility, acrosomal reaction impairment, DNA fragmentation and cell death. This monocentric, randomised, double-blind, placebo-controlled trial investigated the effect of 6 months of supplementation with L-carnitine, acetyl-L-carnitine and other micronutrients on sperm quality in 104 subjects with oligo- and/or astheno- and/or teratozoospermia with or without varicocele. In 94 patients who completed the study, sperm concentration was significantly increased in supplemented patients compared to the placebo ($p = .0186$). Total sperm count also increased significantly ($p = .0117$) in the supplemented group as compared to the placebo group. Both, progressive and total motility were higher in supplemented patients ($p = .0088$ and $p = .0120$, respectively). Although pregnancy rate was not an endpoint of the study, of the 12 pregnancies that occurred during the follow-up, 10 were reported in the supplementation group. In general, all these changes were more evident in varicocele patients. In conclusion, supplementation with metabolic and antioxidant compounds could be efficacious when included in strategies to improve fertility.

KEYWORDS

antioxidant, oligo-astheno-teratozoospermia, sperm, spermogram, varicocele

1 | INTRODUCTION

Infertility is the inability of a sexually active, nonconceiving couple to achieve a spontaneous pregnancy within one year. Worldwide, the incidence of infertility is about 15%, of which, in general, 50% can be attributed to a male-associated factor. This can be reported with or without abnormal semen parameters (WHO, 2000). Male fertility can be affected by many factors ranging from congenital, endocrine,

immunologic, infectious or lifestyle factors as well as various malignancies. On the other hand, in 30%–40% of the cases, no obvious male infertility-associated factor is found (idiopathic male infertility; Nieschlag, Behre, & Nieschlag, 2010).

Varicocele is defined as an abnormal dilatation of scrotal veins, and various studies report a general prevalence of 15% in the healthy male population, whereas it is 40% in infertile men (Nagler, Luntz, & Martinis, 1997). Although the pathophysiologic mechanisms are not

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yet completely known, varicocele has adverse effects on spermatogenesis and, to date, is considered as most common among the known causes of male infertility (Practice Committee of American Society for Reproductive Medicine and the Society for Male Reproduction and Urology, 2014).

Since sperm functions such as capacitation and motility are all highly energy-dependent (Talwar & Hayatnagarkar, 2015), spermatozoa have very high energy requirements. Many factors that negatively affect semen quality act through decreasing energy availability by mitochondrial dysfunction (Amaral, Lourenço, Marques, & Ramalho-Santos, 2013). Spermatozoa are also vulnerable to reactive oxygen species (ROS) because their plasma membranes and cytoplasm are rich in polyunsaturated fatty acids (Agarwal et al., 2014). In particular, elevated ROS exposure leads to membrane damage, membrane instability and functional alterations causing cell death (Agarwal et al., 2014). Latest evidence demonstrates an association between high ROS levels and increased mitochondrial DNA (mtDNA) copy number with decreased mtDNA integrity (Bonanno et al., 2016). Oxidative stress (OS) occurs when there is an imbalance between oxidants and antioxidants (Agarwal, Hamada, & Esteves, 2012). However, for normal sperm cell function including chromatin compaction in maturing spermatozoa during epididymal transit, a delicate redox balance between reduction and oxidation is required (Wright, Milne, & Leeson, 2014). In general, an oxidative milieu may lead to cellular degeneration by apoptosis or necrosis, and a reducing milieu could favour cell survival (Durackova, 2014). Thus, a therapeutic strategy would need to use supplements to increase sperm energy metabolism, minimise free radical damage to sperm and improve the cellular processes connected with the formation and maturation of sperm.

L-Carnitine and acetyl-L-carnitine play an important role in spermatozoa energy metabolism (Agarwal & Said, 2004; Zhou, Liu, & Zhai, 2007). Many clinical studies have shown that oral administration to asthenozoospermic subjects increases the percentage of mobile spermatozoa, progressive rapid motility, average speed and linearity of sperm motility (Balercia et al., 2005; Lenzi et al., 2004). Selenium is an essential component of several major metabolic pathways: antioxidant defence systems, thyroid hormone metabolism and immune function (Brown & Arthur, 2001). Coenzyme Q10 (CoQ10) is concentrated in the mitochondria located in the midpiece of sperm, and the levels of this compound show a significant correlation with sperm count and motility. Furthermore, CoQ10 may be deficient in varicocele leading to higher sensitivity to oxidative damage (Balercia et al., 2004). Fructose, citric acid, vitamin C, vitamin B12 and zinc are related to increased damage to the sperm genetic material, synthesis of coenzymes, metabolism and energy production (Chia, Ong, Chua, Ho, & Tay, 2000; Dawson, Harris, Teter, & Powell, 1992; Moslemi & Tavanbakhsh, 2011).

Thus, the objective of this trial was to evaluate sperm quality after supplementation of oligo- and/or astheno- and/or teratozoospermic subjects with or without varicocele with selected naturally occurring antioxidative compounds in a randomised, double-blind, placebo-controlled setting.

2 | MATERIALS AND METHODS

Between December 2014 and June 2015, 104 infertile patients with oligo- and/or astheno- and/or teratozoospermia with an average age of 32.5 years (range 18–48) were enrolled in this single-centre, randomised, double-blind, placebo-controlled trial to determine the effect of antioxidant supplementation on semen quality. All participants were enrolled from our Andrology Clinic at the Department of Gynecological-Obstetric Sciences and Urological Sciences, “Sapienza” Rome University. The block randomisation method was used to randomise subjects into groups resulting in equal sample sizes to ensure a balance across the groups over time. At the commencement of the study, 52 patients with varicocele grade I-III (confirmed with Doppler ultrasound) and 52 patients without varicocele were divided into two groups each consisting of the supplementation and a placebo subgroup. Ten patients dropped out from the study leaving 45 patients with varicocele and 49 without varicocele.

The supplementation formulation (Proxeed Plus from Sigma-Tau HealthScience, Utrecht, the Netherlands) consisted of 1,000 mg L-carnitine, 725 mg fumarate, 500 mg acetyl-L-carnitine, 1,000 mg fructose, 20 mg CoQ10, 90 mg vitamin C, 10 mg zinc, 200 µg folic acid and 1.5 µg vitamin B12. The placebo was provided from the same company and was made with excipients (sucrose, silica (anti-caking), lemon flavour, acesulfame K (E950) sweetener) of the supplementation without the active compounds.

Subjects received supplements or placebo (two sachets daily for 6 months) according to the randomisation schedule (nQuery Advisor nTerim 2.0 (2012) program) and were instructed of the method of use. One evaluation of a spermogram was carried out at the beginning of the treatment (V1) to examine semen parameters in each patient. At the end of the 6-month treatment (V2), a consecutive semen sample was collected. Together with the semen analyses, before and after the treatment, we collected demographic data (age, weight, height), physical examination, blood pressure, medical history and intake of previous/concomitant therapies.

Semen samples were collected after 3–5 days of sexual abstinence. Ejaculate volume, total sperm count, total and progressive motility, as well as normal sperm morphology were evaluated according to WHO guidelines (2010; 5th edition guidelines).

Subjects included in our trial were men between 18 and 50 years of age with oligo-, astheno- and/or teratozoospermia, with or without varicocele, and having a history of infertility for more than 12 months. The varicocele patients were not surgically treated before and during the treatment. Patients without varicocele were suffering from idiopathic male infertility, and no other previous history of diseases affecting fertility. Every patient underwent a complete check-up to exclude any other cause of infertility (history, examination, complete ultrasound and Doppler, hormones and genetic tests) with no difference between varicocele and nonvaricocele patients. Fertile female partners were required with regular menstrual cycles, age <40 and couples not looking for fertility-related procedures such as *in vitro* fertilization (IVF) or artificial insemination (AI), or intracytoplasmic sperm injection (ICSI) for the next 90 days.

Subjects with known hypersensitivity to any of the treatment compounds, history of undescended testes or cancer, endocrine disorders, history of post-pubertal mumps, genitourinary surgery, obstructive azoospermia or obstructive pathology of the urogenital system, autoimmune disease, cystic fibrosis, history of taking any therapy affecting fertility within last 3 months, excessive consumption of alcohol or regular use of illicit or "recreational" drugs, positive serology for HIV, subjects following any special diet, any condition which in the opinion of the investigator might put the subject at risk by participating in this study and subjects involved in any other clinical trials were excluded from the trial. Endpoints of the study were sperm concentration, semen volume, total sperm count, total motility, progressive motility and percentage of normal sperm morphology.

The Ethical Committee of the Department of Gynecological-Obstetric Sciences and Urological Sciences, "Sapienza" Rome University, approved the study protocol (Institute Ethical Approval Number PXP-001A). The study was conducted in line with European Urology and Good Clinical Practice guidelines, with ethical principles laid down in the latest version of the Declaration of Helsinki. Every patient signed an informed consent to participate in the study.

2.1 | Sample size

Planning to carry out the analysis of covariance in a factorial design with two groups (Proxeed or Placebo, with and without varicocele), defined $f = \sigma_m/\sigma = 0.25$, a correlation coefficient (R^2) between the baseline and final equal to 0.50, an $\alpha = .05$ (significance) and $\beta = .20$ (power of 80%), made it necessary that at least 88 patients equally distributed in 22 units for each subgroup had to be enrolled. However, in anticipation of having about 15% of dropout, 104 patients (52 per arm) were enrolled.

2.2 | Statistical analysis

All continuous variables have been reported as mean, median, standard deviations, minimum and maximum values. Discrete and nominal variables have been reported as frequency and percentage in contingency tables. The basal homogeneity of groups has been tested, on the continuous variables, by the analysis of variance (ANOVA) with two levels (drug and varicocele). The Shapiro-Wilk test was adopted for checking the normal distribution of the data. In the present analysis, no discrete variables were considered for testing the homogeneity of groups.

All the study endpoints considered in the present analysis were evaluated, on the complete sample, by the analysis of covariance for a model with two classification levels. The independent variable was the value detected at the baseline visit, while the dependent variable was the value detected at the end of treatment. The Wilcoxon rank-sum test was adopted for comparing the two groups at baseline, while the Wilcoxon signed rank test was used in the comparisons before/after by group. A "Responder," was defined as, a patient whose parameters improved in comparison to the values before the treatment at the

final visit. A responder analysis was also carried out. The Chi-squared test was adopted for detecting possible differences between the two treatment groups. All the above analyses (apart from the ANCOVA) were repeated for the comparison of the two groups separately by the presence of varicocele. Considering the low power, due to the small size, the responses of the tests presented separately for the presence of the varicocele have to be evaluated accordingly. SAS[®] Vers. 9.4 was used for performing all the analyses.

3 | RESULTS

In total, 94 (of 104) patients completed the study. Table 1 summarises demographic and baseline characteristics of the population by treatment group. The results of the homogeneity tests show that the two groups were well-balanced. The descriptive analyses show that at baseline, all sperm parameters in patients suffering from varicocele were lower when compared to the non-varicocele group.

Adverse events (Table 2) that did not lead to stop the therapy, occurred only in the treatment group. All events were not serious: four patients had nausea and three vertigo or headache.

The results of the inferential analyses of the semen parameters are presented in Table 3. As for the ANCOVA, the p -values refer to the intention-to-treat population (ITT). The last observation carried forward (LOCF) method was used for replacing the missing data. The analyses are also presented for varicocele patients (Table 4) and non-varicocele patients (Table 5). The Wilcoxon rank-sum test was adopted for calculating the p -values. In analyzing the comparisons before/after in the placebo group, a significant difference in some parameters was observed. Therefore, the results of all the tests were also included in both the tables and the text. The responder analysis for all the parameters was carried out for all the groups and included in Table 6.

3.1 | Sperm concentration

The overall results for the sperm concentration in all subjects are summarised in Table 3. In the placebo group, sperm concentration was $41.4 \pm 17.9 \times 10^6/\text{ml}$ at baseline and $43.7 \pm 13.6 \times 10^6/\text{ml}$ at the final visit. In the supplemented group, sperm concentration was $40.8 \pm 18.2 \times 10^6/\text{ml}$ at baseline and $51.4 \pm 13.9 \times 10^6/\text{ml}$ at final visit. While for the placebo group no change was observed ($p = .5244$), the increase in sperm concentration for the treatment group was significant ($p = .0026$). Before the treatment, the sperm concentration in the placebo group did not differ from that in the treatment group ($p = .8453$). At the end of the trial, the difference between both groups was significant ($p = .0186$) in favour of the supplemented group.

In varicocele patients (Table 4), the mean sperm concentrations in the placebo group was $38.7 \pm 18.1 \times 10^6/\text{ml}$ at baseline and $39.9 \pm 17.2 \times 10^6/\text{ml}$ at the final visit ($p = .7572$). In the supplemented

TABLE 1 Baseline characteristics

Parameter	Statistics	Placebo	Supplemented	Total
Age (years)	N	52	52	104
	Missing	0	0	0
	Mean	32.5	32.5	32.5 (<i>p</i> = .9792)
	Std. Deviation	6.7	6.7	6.7
	Median	33.0	32.0	32.3
	Range	19.0–48.6	18.8–48.4	18.8–48.6
Height (cm)	N	52	52	104
	Missing	0	0	0
	Mean	178.6	177.2	177.9 (<i>p</i> = .2487)
	Std. Deviation	6.0	6.0	6.0
	Median	180.0	177.0	178.0
	Range	168.0–190.0	163.0–192.0	163.0–192.0
Weight (kg)	N	52	52	104
	Missing	0	0	0
	Mean	76.4	75.1	75.8 (<i>p</i> = .4242)
	Std. Deviation	8.7	7.7	8.2
	Median	75.0	75.0	75.0
	Range	62.0–94.0	62.0–93.0	62.0–94.0
HR (b/min)	N	52	52	104
	Missing	0	0	0
	Mean	70.8	70.4	70.6 (<i>p</i> = .6295)
	Std. Deviation	4.5	3.5	4.0
	Median	70.0	70.0	70.0
	Range	60–80	60–78	60–80
SBP (mmHg)	N	52	52	104
	Missing	0	0	0
	Mean	119.4	117.2	118.3 (<i>p</i> = .0961)
	Std. Deviation	7.3	6.3	6.9
	Median	120.0	120.0	120.0
	Range	100–130	110–130	100–130
DBP (mmHg)	N	52	52	104
	Missing	0	0	0
	Mean	72.4	73.2	72.8 (<i>p</i> = .4707)
	Std. Deviation	5.8	5.5	5.7
	Median	70.0	70.0	70.0
	Range	60–90	60–85	60–90

Results from ANOVA with two levels (drug and varicocele).

group, sperm concentration significantly (*p* = .0403) increased from $38.5 \pm 19.0 \times 10^6/\text{ml}$ at baseline to $50.2 \pm 17.9 \times 10^6/\text{ml}$ at the final visit. Before the treatment, the placebo and treatment groups showed no difference (*p* = .9708). The comparison of the changes from baseline between the two groups showed nonsignificant difference (*p* = .1391) in favour of the supplemented group.

The results of sperm concentration at baseline in non-varicocele patients (Table 5) in the placebo group were $44.1 \pm 17.5 \times 10^6/\text{ml}$ and $47.2 \pm 7.8 \times 10^6/\text{ml}$ at the final visit (*p* = .5318). In

the supplemented group a mean sperm concentration of $43.2 \pm 17.3 \times 10^6/\text{ml}$ was found at baseline and of $52.5 \pm 9.4 \times 10^6/\text{ml}$ at the final visit (*p* = .0354). Before the treatment, the placebo and treatment groups showed no difference (*p* = .8048). The comparison of the changes from baseline between the two groups showed no difference (*p* = .2460) in favour of the supplemented group.

The responder analysis (Table 6) showed that 73.3% of supplemented patients versus 51.0% of the patients in the placebo group increased from baseline (*p* = .0262).

TABLE 2 Listing of adverse events

Treatment group	Id. no.	Age	Description (PT term)	Seriousness	Relationship	Action taken
Supplemented	36	32	Nausea	Not serious	Probable	None
			Gastro-oesophageal reflux disease	Not serious	Probable	None
	67	27	Nausea	Not serious	Possible	None
			Vertigo	Not serious	Possible	None
	68	21	Headache	Not serious	Possible	None
			Nausea	Not serious	Possible	None
	85	28	Headache	Not serious	Possible	None
Nausea			Not serious	Possible	None	

3.2 | Semen volume

Overall results for the semen volume are summarised in Table 3. While the mean semen volume in the placebo group was 3.0 ± 1.0 ml at baseline, it was 2.9 ± 1.0 ml at the final visit. In the supplemented group, 3.1 ± 1.2 ml were ejaculated at baseline and 3.2 ± 0.9 ml at the final visit. In both groups, placebo and treatment group, no changes ($p = .6787$) and ($p = .6271$) were observed. There were also no differences for the comparison between the two groups at baseline ($p = .7499$) and at the final visit ($p = .1313$).

In varicocele patients (Table 4), the mean semen volume at baseline in the placebo group was 2.7 ± 0.7 ml and 2.4 ± 1.1 ml after the treatment. In the treatment group, semen volume was 2.9 ± 1.2 ml at baseline and 3.2 ± 1.2 ml at the final visit. No difference before/after was observed in both the placebo ($p = .2250$) and the supplemented group ($p = .3632$). Comparing the two groups at baseline ($p = .8761$) and at the end of the study showed also no difference ($p = .1273$).

As for non-varicocele patients (Table 5), the semen volume in the placebo group was 3.2 ± 1.1 ml before and 3.3 ± 0.8 ml after the treatment. In the supplemented group it was 3.4 ± 1.2 ml at baseline and 3.3 ± 0.6 ml at the final visit. Furthermore, for non-varicocele patients, no difference before/after was observed in both the placebo group ($p = .7711$) and the supplemented group ($p = .8753$). The data at baseline ($p = .6144$) and at the end of the study ($p = .5026$) also did not differ.

The responder analysis (Table 6) did not show a difference between the two groups; 48.9% of supplemented patients versus 46.9% in the placebo group were considered as responders at final visit ($p = .8500$).

3.3 | Total sperm count

The overall results for the total sperm count in all subjects are summarised in Table 3. In the placebo group, $113.1 \pm 37.4 \times 10^6$ at baseline and $127.8 \pm 61.4 \times 10^6$ at the final visit, while the total sperm count in the supplemented group, was $114.2 \pm 37.8 \times 10^6$ at baseline and $163.5 \pm 64.3 \times 10^6$ at the final visit. While for the placebo group no change was observed ($p = .2030$), the increase in the supplemented group was highly significant ($p < .0001$). In contrast,

no difference ($p = .8658$) was observed between the two groups at baseline. At the end of the study, the two groups differed in favour of the supplemented group as was confirmed by the inferential analysis with $p = .0117$.

In the varicocele group (Table 4), total sperm count in the placebo was $100.5 \pm 41.9 \times 10^6$ at baseline and $102.4 \pm 77.2 \times 10^6$ at the final visit ($p = .5749$). The supplemented group had a sperm concentration of $96.3 \pm 36.1 \times 10^6$ at baseline and of $158.8 \pm 90.1 \times 10^6$ at the final visit ($p = .0009$). While both groups did not differ at baseline ($p = .8764$), the values in the supplemented group were significantly higher at the final visit ($p = .0066$).

In non-varicocele patients (Table 5), the total sperm count for the group was $125.6 \pm 27.7 \times 10^6$ at baseline and $152.1 \pm 23.9 \times 10^6$ after the treatment ($p = .0022$) and increased significantly ($p = .0005$), from $132.0 \pm 30.9 \times 10^6$ at baseline to $167.6 \pm 28.5 \times 10^6$ at final visit in the supplemented groups. No significant difference between the two groups was observed at baseline ($p = .4259$) and final visit ($p = .2460$).

The responder analysis (Table 6) showed that 82.2% of supplemented patients versus 55.1% in the placebo group increased from baseline ($p = .0048$).

3.4 | Progressive motility

For the progressive motility, the overall results are summarised in Table 3. In the placebo group, progressive motility was $23.0 \pm 7.8\%$ at baseline and $24.5 \pm 7.2\%$ at the final visit ($p = .1567$), while it was $23.4 \pm 6.1\%$ and $28.6 \pm 8.2\%$ at baseline final visit ($p = .0012$). The two groups did not differ ($p = .6701$), before the treatment. However, at the end of the study, a significant ($p = .0088$) difference in favour of the supplemented group was observed.

In varicocele patients (Table 4), progressive motility was $21.8 \pm 6.2\%$ at baseline and $23.6 \pm 6.8\%$ at the final visit in the placebo group ($p = .1570$) while it was $23.1 \pm 5.2\%$ and $27.4 \pm 7.2\%$, respectively visit in the treated group ($p = .0149$). No difference between the two groups was observed at baseline ($p = .3096$) and at study end ($p = .1686$).

In the non-varicocele group (Table 5), progressive motility was $24.2 \pm 9.1\%$ before and $25.4 \pm 7.7\%$ after treatment in the placebo arm ($p = .4866$) and $23.7 \pm 7.0\%$ at baseline and $29.6 \pm 9.0\%$ at the final visit in the treatment arm ($p = .0311$). Also for this comparison,

the test was not statistically significant for both baseline ($p = .8907$) and final visit ($p = .2040$).

The responder analysis (Table 6) showed that 73.3% of supplemented patients responded to the treatment whereas it was only 51.0% in the placebo group ($p = .0262$).

3.5 | Total motility

The summary results for total motility are depicted in Table 3. In the placebo group, the means were $32.6 \pm 9.2\%$ before and $34.6 \pm 7.1\%$ after the treatment ($p = .1483$). In the supplemented group, baseline and

TABLE 3 Sperm parameters. Absolute values of baseline and final

Parameter	Groups	Statistics	Baseline	Final	<i>p</i> -Values before/after**
Sperm concentration (10^6 ml)	Placebo	N	52	49	.5244
		Missing	0	3	
		Mean	41.4	43.7	
		Std. Deviation	17.9	13.6	
		Median	38.3	44.0	
		Range	11.0; 79.0	16.0; 79.0	
	Supplemented	N	52	45	.0026
		Missing	0	7	
		Mean	40.8	51.4	
		Std. Deviation	18.2	13.9	
		Median	39.0	49.0	
		Range	12.3; 77.0	28.0; 86.0	
		<i>p</i> -Values by visit*	.8453	.0186	
Volume of ejaculate (ml)	Placebo	N	52	49	.6787
		Missing	0	3	
		Mean	3.0	2.9	
		Std. Deviation	1.0	1.0	
		Median	2.8	3.0	
		Range	1.3; 6.3	1.1; 5.1	
	Supplemented	N	52	45	.6271
		Missing	0	7	
		Mean	3.1	3.2	
		Std. Deviation	1.2	0.9	
		Median	2.8	3.2	
		Range	1.4; 6.0	1.1; 5.5	
		<i>p</i> -Values by visit*	.7499	.1313	
Total sperm count (10^6)	Placebo	N	52	49	.2030
		Missing	0	3	
		Mean	113.1	127.8	
		Std. Deviation	37.4	61.4	
		Median	107.6	136.7	
		Range	30.0; 197.6	24.0; 270.0	
	Supplemented	N	52	45	<.0001
		Missing	0	7	
		Mean	114.2	163.5	
		Std. Deviation	37.8	64.3	
		Median	112.1	158.4	
		Range	43.2; 205.8	48.4; 369.6	
		<i>p</i> -Values by visit*	.8658	.0117	

(Continues)

TABLE 3 (Continued)

Parameter	Groups	Statistics	Baseline	Final	p-Values before/after**
Progressive motility (%)	Placebo	N	52	49	.1567
		Missing	0	3	
		Mean	23.0	24.5	
		Std. Deviation	7.8	7.2	
		Median	22.3	23.0	
		Range	5.9; 43.2	8.1; 44.0	
	Supplemented	N	52	45	.0012
		Missing	0	7	
		Mean	23.4	28.6	
		Std. Deviation	6.1	8.2	
		Median	23.2	27.0	
		Range	12.0; 40.0	15.0; 57.9	
		p-Values by visit*	.6701	.0088	
Total motility (%)	Placebo	N	52	49	.1483
		Missing	0	3	
		Mean	32.6	34.6	
		Std. Deviation	9.2	7.1	
		Median	32.0	35.0	
		Range	8.0; 55.0	12.0; 49.2	
	Supplemented	N	52	45	<.0001
		Missing	0	7	
		Mean	31.7	39.0	
		Std. Deviation	8.2	8.0	
		Median	31.3	37.5	
		Range	18.9; 48.0	29.0; 65.3	
		p-Values by visit*	.5239	.0120	
Sperm morphology—typical (%)	Placebo	N	52	49	.0146
		Missing	0	3	
		Mean	21.1	15.7	
		Std. Deviation	16.2	9.4	
		Median	15.0	15.0	
		Range	3.0; 59.0	3.0; 52.0	
	Supplemented	N	52	44	.0055
		Missing	0	8	
		Mean	23.5	17.7	
		Std. Deviation	14.6	15.2	
		Median	20.0	13.5	
		Range	5.0; 64.0	3.0; 77.0	
		p-Values by visit*	.2062	.3791	

(Continues)

values obtained at the final visit differed significantly ($p < .0001$). While the two groups were balanced at baseline ($p = .5239$), a significant ($p = .0120$) difference between the two groups at the end of the treatment was evident in favour of the supplemented group.

In varicocele patients (Table 4), total sperm motility in subjects who received the placebo did not change from baseline to the final visit ($p = .1214$). However, in the treated group, values increased from $31.5 \pm 8.1\%$ at baseline to $37.5 \pm 7.1\%$ after the treatment ($p = .0065$).

TABLE 3 (Continued)

Parameter	Groups	Statistics	Baseline	Final	<i>p</i> -Values before/after**	
Sperm morphology—atypical (%)	Placebo	<i>N</i>	52	49	.0105	
		Missing	0	3		
		Mean	78.9	84.1		
		Std. Deviation	15.4	9.3		
		Median	82.5	85.0		
		Range	41.0; 97.0	48.0; 97.0		
	Supplemented	<i>N</i>	52	45	.1310	
		Missing	0	7		
		Mean	80.2	82.5		
		Std. Deviation	16.6	15.2		
		Median	85.0	86.0		
		Range	22.0; 96.0	23.0; 100.0		
		<i>p</i> -Values by visit*		.5379		.5081

*The *p*-values for the Baseline visit are derived from the comparison between the two groups with the Wilcoxon rank-sum test. The *p*-values for the final visit are derived from the ANCOVA on the ITT population.

**The *p*-values before/after by treatment group are derived from the Wilcoxon signed rank test.

Yet, no difference was observed at baseline ($p = .7836$) and at the end of the study ($p = .3164$).

As for the non-varicocele group (Table 5), total motility was $33.9 \pm 10.2\%$ before and $34.7 \pm 7.5\%$ after the treatment in the placebo group ($p = .5604$) and $31.8 \pm 8.4\%$ and $40.2 \pm 8.7\%$ at baseline and final visit respectively, in the treated group ($p = .0028$). Both groups were balanced at baseline ($p = .5396$). However a statistical difference in favour of the supplemented group was evident ($p = .0257$).

The responder analysis (Table 6) showed that 68.9% of supplemented patients versus 53.1% in the placebo group increased from baseline ($p = .1166$).

3.6 | Normal sperm morphology

Analyzing sperm morphology (Table 3), results indicate that in the placebo group $21.1 \pm 16.2\%$ sperm at baseline and $15.7 \pm 9.4\%$ sperm after the treatment ($p = .0146$) has normal morphology. In the treatment group, normal sperm morphology was $23.5 \pm 14.6\%$ before and $17.7 \pm 15.2\%$ after the ($p = .0055$) treatment. No difference between the two groups was observed at baseline ($p = .2062$) and at study end ($p = .3791$).

Looking at atypical morphology (Table 3) the placebo group showed significantly ($p = .0105$) higher values at the final visit, while baseline and final values in the supplemented group did not differ ($p = .1310$). Further, two groups did not differ at baseline ($p = .5379$) and at the end of study ($p = .5081$).

3.7 | Pregnancy rate

Twelve pregnancies occurred during the follow-up time: 10 in the supplementation group (nine non-varicocele and one varicocele) and

two in the placebo group (one non-varicocele and one varicocele). One spontaneous abortion was reported in the placebo arm.

4 | DISCUSSION

Male infertility is a medical condition and a relevant social problem that has a strong impact on well-being. From various studies, it has emerged that seminal oxidative stress and sperm DNA damage must be taken into account as critical factors in the aetiology of semen alterations and infertility (Saalu, 2010; Vessey et al., 2016).

When levels of free radicals, and in particular ROS, are increased and antioxidant levels are decreased, OS occurs (Agarwal, Roychoudhury, Bjugstad, & Chou, 2016). OS has negative effects on sperm quality parameters and was shown to impact the DNA carried by these specialised cells. Human mitochondrial DNA gene alterations were associated with many pathological conditions, and this damage per se is also a recognized cause of poor sperm quality. Thus, targeting OS is a strategy to increase fertility and spermatozoa number and quality (Agarwal et al., 2016).

Varicocele is associated with an increase in ROS production and seminal OS leading to sperm dysfunction. Mitochondria are a key source of ROS production especially when they are damaged or dysfunctional due to lack of proper substrates and cofactors. In addition, mitochondrial gene mutations can also affect the respiratory electron transfer chain. These DNA variants probably underlie mitochondrial dysfunction leading to impaired ATP synthesis and ultimately interfere with sperm motility and fertility status (Heidari et al., 2016).

Non-enzymatic antioxidants including vitamins (mainly vitamins A, B, C, E), glutathione as well as metabolic coenzymes such as pantothenic acid, Co Q10, carnitines (L-carnitine and acetyl-L-carnitine)

and micronutrients (zinc, selenium, copper) are often deficient, hence causing a general diminution in the antioxidant status as well as mitochondrial dysfunction (Jeulin & Lewin, 1996; Virmani, Ali, Pinto, Zerelli, & Binienda, 2016). Nutrients such as zinc, folic acid, vitamin B12, L-carnitine and acetyl-L-carnitine are also associated with sperm

production and maturation (Adams et al., 1998; Ebisch, Thomas, Peters, Braat, & Steegers-Theunissen, 2007; Jeulin & Lewin, 1996; Watanabe et al., 2003).

Studies demonstrate that using these substances has a beneficial effect on fertility, in particular on sperm quality and are therefore

TABLE 4 Sperm parameters. Patients with varicocele. Absolute values of baseline and final visits

Parameter	Groups	Statistics	Baseline	Final	p-Values before/after**
Sperm concentration (10 ⁶ ml)	Placebo	N	26	24	
		Missing	0	2	
		Mean	38.7	39.9	.7572
		Std. Deviation	18.1	17.2	
		Median	32.0	34.8	
		Range	17.5; 76.0	16.0; 79.0	
	Supplemented	N	26	21	
		Missing	0	5	
		Mean	38.5	50.2	.0403
		Std. Deviation	19.0	17.9	
		Median	32.5	45.0	
		Range	12.3; 76.0	28.0; 86.0	
		p-Values by visit*	.9708	.1391	
Volume of ejaculate (ml)	Placebo	N	26	24	
		Missing	0	2	
		Mean	2.7	2.4	.2250
		Std. Deviation	0.7	1.1	
		Median	2.7	2.2	
		Range	1.3; 4.1	1.1; 5.0	
	Supplemented	N	26	21	
		Missing	0	5	
		Mean	2.9	3.2	.3632
		Std. Deviation	1.2	1.2	
		Median	2.6	3.2	
		Range	1.4; 5.6	1.1; 5.5	
		p-Values by visit*	.8761	.1273	
Total sperm count (10 ⁶)	Placebo	N	26	24	
		Missing	0	2	
		Mean	100.5	102.4	.5749
		Std. Deviation	41.9	77.2	
		Median	94.9	77.7	
		Range	30.0; 197.6	24.0; 270.0	
	Supplemented	N	26	21	
		Missing	0	5	
		Mean	96.3	158.8	.0009
		Std. Deviation	36.1	90.1	
		Median	96.1	126.0	
		Range	43.2; 190.6	48.4; 369.6	
		p-Values by visit*	.8764	.0066	

(Continues)

TABLE 4 (Continued)

Parameter	Groups	Statistics	Baseline	Final	<i>p</i> -Values before/after**
Progressive motility (%)	Placebo	N	26	24	.1570
		Missing	0	2	
		Mean	21.8	23.6	
		Std. Deviation	6.2	6.8	
		Median	22.3	22.0	
		Range	10.0; 40.0	15.0; 44.0	
	Supplemented	N	26	21	.0149
		Missing	0	5	
		Mean	23.1	27.4	
		Std. Deviation	5.2	7.2	
		Median	23.4	27.0	
		Range	13.0; 33.3	15.0; 41.7	
		<i>p</i> -Values by visit*	.3096	.1686	
Total motility (%)	Placebo	N	26	24	.1214
		Missing	0	2	
		Mean	31.3	34.5	
		Std. Deviation	8.2	6.9	
		Median	31.5	35.0	
		Range	8.0; 45.0	18.0; 49.0	
	Supplemented	N	26	21	.0065
		Missing	0	5	
		Mean	31.5	37.5	
		Std. Deviation	8.1	7.1	
		Median	30.4	36.0	
		Range	21.0; 46.7	29.0; 55.0	
		<i>p</i> -Values by visit*	.7836	.3164	

*The *p*-values for the Baseline visit are derived from the comparison between the actual baseline values of the two groups with the Wilcoxon rank-sum test. The *p*-values for the final visit are derived from the comparison on the differences before/after between the two groups with the Wilcoxon rank-sum test.

**The *p*-values before/after by treatment group are derived from the Wilcoxon signed rank test.

recommended as potentially effective therapy for the treatment of male infertility (Walczak-Jedrzejowska, Wolski, & Slowikowska-Hilczler, 2013).

L-Carnitine together with acetyl-L-carnitine is a safe treatment commonly used because of their capacity in improving sperm quality and pregnancy rate in males suffering from astheno-teratozoospermia (Wang et al., 2010). Selenium is essential for testis development, spermatogenesis and final sperm quality. It acts via a positive antioxidant action through glutathione peroxidase enzymes (Moslemi & Tavanbakhsh, 2011). Both vitamin E and zinc play a role in antioxidant balance regulation and are able to improve sperm concentration, percentage of progressively motile sperm and consequently pregnancy rate (Ajina, Sallem, Haouas, & Mehdi, 2016; Chen et al., 2012). Coenzyme Q10 levels show a significant correlation with sperm count and with sperm motility (Festa et al., 2014; Mancini et al., 1994). Administration of coenzyme Q10 to men with idiopathic asthenozoospermia results in an increase in sperm motility (Balercia et al., 2004).

The real association between varicocele and fertility status is still not completely clarified, but a recent meta-analysis showed a significant improvement in semen parameters in patients after varicocelectomy (Agarwal et al., 2007). Furthermore, after surgical treatment, a reversal in the sperm DNA damage was evidenced (Zini & Dohle, 2011). Gual-Frau et al. (2015) confirmed a beneficial effect of antioxidant compounds in patients suffering from grade I varicocele. In their study, patients showed an average relative reduction of 22.1% in sperm DNA fragmentation ($p = .02$) with 31.3% fewer highly degraded sperm cells ($p = .07$). The total number of sperm was also significantly increased after 3 months of treatment. Another recent trial conducted on patients with high-grade varicocele, and randomised for surgical treatment or L-carnitine supplementation, reported good results in all sperm parameters: motility changed from 21.7% to 35.4% (vs. 33.9%–47.5% in L-carnitine group), normal sperm morphology changed from 46.3% to 60% (vs. 56.6%–69.7% in the L-carnitine group) and seminal volume changed

from 3.5 to 4.2 ml (vs. 2.9–4.3 ml in the L-carnitine group). The authors concluded that supplementary treatment was as effective as varicocele surgery in improving semen parameters and can therefore be used as an alternative to surgery (Sofimajidpour, Ghaderi, & Ganji, 2016).

Lastly, in a Cochrane analysis, men taking oral dietary supplementation for infertility were able to obtain better live birth rates in couples undergoing assisted reproductive techniques (Showell et al., 2014).

Comparing the effectiveness of varicocele surgery and medical therapy to treat these cases of infertility is difficult, and trials are

TABLE 5 Sperm parameters. Patients without varicocele. Absolute values of baseline and final visits

Parameter	Groups	Statistics	Baseline	Final	p-Values before/after**
Sperm concentration (10 ⁶ ml)	Placebo	N	26	25	.5318
		Missing	0	1	
		Mean	44.1	47.2	
		Std. Deviation	17.5	7.8	
		Median	42.3	48.5	
		Range	11.0; 79.0	30.0; 65.0	
	Supplemented	N	26	24	.0354
		Missing	0	2	
		Mean	43.2	52.5	
		Std. Deviation	17.3	9.4	
		Median	42.4	50.5	
		Range	19.0; 77.0	37.7; 78.0	
		p-Values by visit*	.8048	.2460	
Volume of ejaculate(ml)	Placebo	N	26	25	.7711
		Missing	0	1	
		Mean	3.2	3.3	
		Std. Deviation	1.1	0.8	
		Median	3.0	3.1	
		Range	1.5; 6.3	2.0; 5.1	
	Supplemented	N	26	24	.8753
		Missing	0	2	
		Mean	3.4	3.3	
		Std. Deviation	1.2	0.6	
		Median	3.3	3.2	
		Range	1.9; 6.0	2.0; 4.5	
		p-Values by visit*	.6144	.5026	
Total sperm count (10 ⁶)	Placebo	N	26	25	.0022
		Missing	0	1	
		Mean	125.6	152.1	
		Std. Deviation	27.7	23.9	
		Median	118.0	154.0	
		Range	69.3; 178.6	107.5; 200.9	
	Supplemented	N	26	24	.0005
		Missing	0	2	
		Mean	132.0	167.6	
		Std. Deviation	30.9	28.5	
		Median	132.2	163.8	
		Range	68.4; 205.8	107.5; 220.8	
		p-Values by visit*	.4259	.2460	

(Continues)

TABLE 5 (Continued)

Parameter	Groups	Statistics	Baseline	Final	<i>p</i> -Values before/after**
Progressive motility (%)	Placebo	N	26	25	.4866
		Missing	0	1	
		Mean	24.2	25.4	
		Std. Deviation	9.1	7.7	
		Median	23.9	25.0	
		Range	5.9; 43.2	8.1; 40.0	
	Supplemented	N	26	24	.0311
		Missing	0	2	
		Mean	23.7	29.6	
		Std. Deviation	7.0	9.0	
		Median	23.2	27.5	
		Range	12.0; 40.0	15.0; 57.9	
		<i>p</i> -Values by visit*	.8907	.2040	
Total motility (%)	Placebo	N	26	25	.5604
		Missing	0	1	
		Mean	33.9	34.7	
		Std. Deviation	10.2	7.5	
		Median	32.0	35.0	
		Range	15.5; 55.0	12.0; 49.2	
	Supplemented	N	26	24	.0028
		Missing	0	2	
		Mean	31.8	40.2	
		Std. Deviation	8.4	8.7	
		Median	31.4	37.8	
		Range	18.9; 48.0	29.0; 65.3	
		<i>p</i> -Values by visit*	.5396	.0257	

*The *p*-values for the Baseline visit are derived from the comparison between the actual baseline values of the two groups with the Wilcoxon rank-sum test. The *p*-values for the final visit are derived from the comparison on the differences before/after between the two groups with the Wilcoxon rank-sum test.

**The *p*-values before/after by treatment group are derived from the Wilcoxon signed rank test.

limited by small case studies and non-randomisation. There is only one report with a direct comparison between L-carnitine and varicocelectomy in patients with grade II/III varicocele. The authors describe a statistically significant improvement in sperm count, motility and morphology after treatment, and results are not different between different treatment methods. The main limitations of the study are the inclusion criteria, small sample size. In addition, this study is not a randomized, double-blind placebo-controlled (DBPC) study (Sofimajidpour et al., 2016).

Our trial evaluated the utilisation of a combination of metabolic substances, antioxidants and micronutrients to improve sperm parameters. For a better understanding of the action of the supplementation, we applied a DBPC system and very specific inclusion and exclusion criteria. Furthermore, in consideration of the still not clear effect of varicocele on male fertility, we divided our cohort into infertile varicocele patients and idiopathic infertile non-varicocele patients. At the end of the trial, we observed a marked increase in

sperm count and concentration together with increases in motility, progressive motility and morphology. All differences between treatment and placebo groups were statistically significant in both varicocele and non-varicocele patients. A small difference (not statistically significant) was also observed in the semen volume in favour of the experimental group. In general, differences were more evident in those patients suffering from varicocele, which can probably be explained with the major OS and ROS-mediated damage that is usually associated with this condition. Unfortunately, at the moment, it is not possible to conclude whether the medical treatment is inferior or superior to varicocelectomy in those men with varicocele. Affirmation whether or not oral supplementation can replace surgery has yet to be properly established. Nevertheless, it is important to take into consideration that the role of oral supplements in clinical practice in the two groups is completely different, and one could possibly rather speak about an association between surgery and oral supplementation be more appropriate.

TABLE 6 Responder analysis

Parameter	Placebo		Supplemented		p-Values
	Non responders	Responders	Non responders	Responders	
Sperm concentration (10 ⁶ ml)	24 (49.0%)	25 (51.0%)	12 (26.7%)	33 (73.3%)	.0262
W varicocele	12 (50.0%)	12 (50.0%)	6 (28.6%)	15 (71.4%)	.1432
W/O varicocele	12 (48.0%)	13 (52.0%)	6 (25.0%)	18 (75.0%)	.0950
Volume of ejaculate (ml)	26 (53.1%)	23 (46.9%)	23 (51.1%)	22 (48.9%)	.8500
W varicocele	16 (66.7%)	8 (33.3%)	9 (42.9%)	12 (57.1%)	.1088
W/O varicocele	10 (40.0%)	15 (60.0%)	14 (58.3%)	10 (41.7%)	.1994
Total sperm count (10 ⁶)	22 (44.9%)	27 (55.1%)	8 (17.8%)	37 (82.2%)	.0048
W varicocele	15 (62.5%)	9 (37.5%)	4 (19.0%)	17 (81.0%)	.0032
W/O varicocele	7 (28.0%)	18 (72.0%)	4 (16.7%)	20 (83.3%)	.3419
Progressive motility (%)	24 (49.0%)	25 (51.0%)	12 (26.7%)	33 (73.3%)	.0262
W varicocele	12 (50.0%)	12 (50.0%)	5 (23.8%)	16 (76.2%)	.0706
W/O varicocele	12 (48.0%)	13 (52.0%)	7 (29.2%)	17 (70.8%)	.1762
Total motility (%)	23 (46.9%)	26 (53.1%)	14 (31.1%)	31 (68.9%)	.1166
W varicocele	11 (45.8%)	13 (54.2%)	7 (33.3%)	14 (66.7%)	.3932
W/O varicocele	12 (48.0%)	13 (52.0%)	7 (29.2%)	17 (70.8%)	.1762

Although pregnancy rate was not an endpoint of the study, it is interesting to note that of the 12 pregnancies that occurred during the follow-up time, 10 were reported in the supplementation group.

The safety of the formulation was assured by its composition, and tolerability was confirmed by the almost total absence of adverse effects during the treatment. We did not compare the effect of this treatment with surgical treatment of varicocele, and we did not evaluate DNA fragmentation and the levels of ROS. Furthermore, latest evidences revealed that evaluating OS can be a diagnostic tool in predicting the best responders to supplementation (Vessey et al., 2016). Oxidative stress is a cause of male infertility with significant negative effect on semen parameters, and varicocele is not only causing OS, but also an additional cause of poor sperm quality. The use of carnitines and other functional substances can form part of an efficacious strategy to manage and treat male infertility in both non-varicocele and varicocele subjects. Indeed, we plan future studies to examine the role of energy metabolism, OS and ROS in particular to gain a better understanding of underlying mechanisms and thereby to determine the best strategies for male infertility treatment.

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REFERENCES

Adams, S. H., Esser, V., Brown, N. F., Ing, N. H., Johnson, L., Foster, D. W., & McGarry, J. D. (1998). Expression and possible role of muscle-type

carnitine palmitoyltransferase I during sperm development in the rat. *Biology of Reproduction*, 59(6), 1399–1405. <https://doi.org/10.1095/biolreprod59.6.1399>

Agarwal, A., Deepinder, F., Cocuzza, M., Agarwal, R., Short, R. A., Sabanegh, E., & Marmar, J. L. (2007). Efficacy of varicolectomy in improving semen parameters: New meta-analytical approach. *Urology*, 70(3), 532–538. <https://doi.org/10.1016/j.urology.2007.04.011>

Agarwal, A., Hamada, A., & Esteves, S. C. (2012). Insight into oxidative stress in varicocele-associated male infertility: Part 1. *Nature Reviews Urology*, 9, 678–690. <https://doi.org/10.1038/nrurol.2012.197>

Agarwal, A., Mulgund, A., Alshahrani, S., Assidi, M., Abuzenadah, A. M., Sharma, R., & Sabanegh, E. (2014). Reactive oxygen species and sperm DNA damage in infertile men presenting with low level leukocytospermia. *Reproductive Biology and Endocrinology*, 12, 126. <https://doi.org/10.1186/1477-7827-12-126>

Agarwal, A., Roychoudhury, S., Bjugstad, K. B., & Chou, C. L. (2016). Oxidation-reduction potential of semen: What is its role in the treatment of male infertility? *Therapeutic Advances in Urology*, 8(5), 302–318. <https://doi.org/10.1177/1756287216652779>

Agarwal, A., & Said, T. M. (2004). Carnitines and male infertility. *Reproductive Biomedicine Online*, 8(4), 376–384. [https://doi.org/10.1016/S1472-6483\(10\)60920-0](https://doi.org/10.1016/S1472-6483(10)60920-0)

Ajina, T., Sallem, A., Haouas, Z., & Mehdi, M. (2016). Total antioxidant status and lipid peroxidation with and without in vitro zinc supplementation in infertile men. *Andrologia*, 49, e127.

Amaral, A., Lourenço, B., Marques, M., & Ramalho-Santos, J. (2013). Mitochondria functionality and sperm quality. *Reproduction*, 146(5), R163–R174. <https://doi.org/10.1530/REP-13-0178>

Balercia, G., Mosca, F., Mantero, F., Boscaro, M., Mancini, A., Ricciardo-Lamonica, G., & Littera, G. P. (2004). Coenzyme Q(10) supplementation in infertile men with idiopathic asthenozoospermia: An open, uncontrolled pilot study. *Fertility and Sterility*, 81(1), 93–98. <https://doi.org/10.1016/j.fertnstert.2003.05.009>

Balercia, G., Regoli, F., Armeni, T., Koverech, A., Mantero, F., & Boscaro, M. (2005). Placebo-controlled double blind randomized trial on the use of l-carnitine, l-acetylcarnitine or combined l-carnitine and l-acetylcarnitine

- in idiopathic asthenozoospermia. *Fertility and Sterility*, 84(3), 662–671. <https://doi.org/10.1016/j.fertnstert.2005.03.064>
- Bonanno, O., Rome, G., Asero, P., Pezzino, F. M., Castiglione, R., Burrello, N., ... D'Agata, R. (2016). Sperm of patients with severe asthenozoospermia show biochemical, molecular and genomic alterations. *Reproduction*, 152(6), 695–704. <https://doi.org/10.1530/REP-16-0342>
- Brown, K. M., & Arthur, J. R. (2001). Selenium, selenoproteins and human health: A review. *Public Health Nutrition*, 4(2B), 593–599.
- Chen, X. F., Li, Z., Ping, P., Dai, J. C., Zhang, F. B., & Shang, X. J. (2012). Efficacy of natural vitamin E on oligospermia and asthenospermia: A prospective multi-centered randomized controlled study of 106 cases. *Azhonghua Nan Ke Xue*, 18(5), 428–431.
- 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(1), 53–57.
- Dawson, E. B., Harris, W. A., Teter, M. C., & Powell, L. C. (1992). Effect of ascorbic acid supplementation on the sperm quality of smokers. *Fertility and Sterility*, 58(5), 1034–1039. [https://doi.org/10.1016/S0015-0282\(16\)55456-9](https://doi.org/10.1016/S0015-0282(16)55456-9)
- Durackova, Z. (2014). Free radicals and antioxidants for non-experts. In I. Laher (Ed.), *Systems biology of free radicals and antioxidants* (pp. 537–565). Berlin, Germany: Springer Verlag.
- Ebisch, I. M., Thomas, C. M., Peters, W. H., Braat, D. D., & Steegers-Theunissen, R. P. (2007). The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. *Human Reproduction Update*, 13(2), 163–174. <https://doi.org/10.1093/humupd/dml054>
- Festa, R., Giacchi, E., Raimondo, S., Tiano, L., Zuccarelli, P., Silvestrini, A., ... Mancini, A. (2014). Coenzyme Q10 supplementation in infertile men with low-grade varicocele: An open, uncontrolled pilot study. *Andrologia*, 46(7), 805–807. <https://doi.org/10.1111/and.2014.46.issue-7>
- Gual-Frau, J., Abad, C., Amengual, M. J., Hannaoui, N., Checa, M. A., Ribas-Maynou, J., ... Prats, J. (2015). Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade I varicocele patients. *Human Fertility*, 18(3), 225–229. <https://doi.org/10.3109/14647273.2015.1050462>
- Heidari, M. M., Khatami, M., Danafar, A., Dianat, T., Farahmand, G., & Talebi, A. R. (2016). Mitochondrial genetic variation in Iranian infertile men with varicocele. *International Journal of Fertility & Sterility*, 10(3), 303–309.
- 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(2), 87–102. <https://doi.org/10.1093/humupd/2.2.87>
- Lenzi, A., Sgro, P., Salacone, P., Paoli, D., Gilio, B., Lombardo, F., ... Gandini, L. (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(6), 1578–1584. <https://doi.org/10.1016/j.fertnstert.2003.10.034>
- Mancini, A., De Marinis, L., Oradei, A., Hallgass, M. E., Conte, G., Pozza, D., & Littarru, G. P. (1994). Coenzyme Q10 concentrations in normal and pathological human seminal fluid. *Journal of Andrology*, 15(6), 591–594.
- Moslemi, M. K., & Tavanbakhsh, S. (2011). Selenium-vitamin E supplementation in infertile men: Effects on semen parameters and pregnancy rate. *International Journal of General Medicine*, 23(4), 99–104. <https://doi.org/10.2147/IJGM>
- Nagler, H. M., Luntz, R. K., & Martinis, F. G. (1997). Varicocele. In L. I. Lipshultz & S. S. Howards (Eds.), *Infertility in the male* (pp. 336–359). St. Louis, MO: Mosby Year Book.
- Nieschlag, E., Behre, H. M., & Nieschlag, S. (2010). *Male reproductive health and dysfunction*. Andrology. Berlin, Germany: Springer Verlag.
- Practice Committee of the American Society for Reproductive Medicine and the Society for Male Reproduction and Urology (2014). Report on varicocele and infertility: A committee opinion. *Fertility and Sterility*, 102(6), 1556–1560.
- Saalu, L. C. (2010). The incriminating role of reactive oxygen species in idiopathic male infertility: An evidence based evaluation. *Pakistan Journal of Biological Sciences*, 13(9), 413–422. <https://doi.org/10.3923/pjbs.2010.413.422>
- Showell, M. G., Mackenzie-Proctor, R., Brown, J., Yazdani, A., Stankiewicz, M. T., & Hart, R. J. (2014). Antioxidants for male subfertility. *Cochrane Database Systematic Review*, (12), CD007411.
- Sofimajidpour, H., Ghaderi, E., & Ganji, O. (2016). Comparison of the effects of varicocele and oral L-carnitine on sperm parameters in infertile men with varicocele. *Journal of Clinical and Diagnostic Research*, 10(4), PC07–PC10.
- Talwar, P., & Hayatnagarkar, S. (2015). Sperm function test. *Journal of Human Reproductive Sciences*, 8(2), 61–69. <https://doi.org/10.4103/0974-1208.158588>
- Vessey, W., McDonald, C., Virmani, A., Almeida, P., Jayasena, C., & Ramsay, J. (2016). Levels of reactive oxygen species (ROS) in the seminal plasma predicts the effectiveness of L-carnitine to improve sperm function in men with infertility. *Endocrine Abstracts* 44, P232.
- Virmani, A., Ali, S., Pinto, L., Zerelli, S., & Binienda, Z. (2016). Genomic effects of food bioactives in neuroprotection. In M. Kussmann & P. Stover (Eds.), *Nutrigenomics and proteomics in health and disease: Towards a systems-level understanding of gene-diet interactions* (pp. 156–165). Chichester, UK: Wiley & Sons, Ltd.
- Walczak-Jedrzejowska, R., Wolski, J. K., & Slowikowska-Hilczner, J. (2013). The role of oxidative stress and antioxidants in male fertility. *Central European Journal of Urology*, 66(1), 60–67. <https://doi.org/10.5173/cej.2013.01>
- Wang, Y. X., Yang, S. W., Qu, C. B., Huo, H. X., Li, W., Li, J. D., ... Cai, G. Z. (2010). L-carnitine: Safe and effective for asthenozoospermia. *Zhonghua Nan Ke Xue*, 16(5), 420–422.
- Watanabe, T., Ohkawa, K., Kasai, S., Ebara, S., Nakano, Y., & Watanabe, Y. (2003). The effects of dietary vitamin B12 deficiency on sperm maturation in developing and growing male rats. *Congenital Anomalies*, 43(1), 57–64. <https://doi.org/10.1111/cga.2003.43.issue-1>
- WHO (2000). *WHO manual for the standardized investigation and diagnosis of the infertile couple*. Cambridge, UK: Cambridge University Press.
- Wright, C., Milne, S., & Leeson, H. (2014). Sperm DNA damage caused by oxidative stress: Modifiable clinical, lifestyle and nutritional factors in male infertility. *Reproductive Biomedicine Online*, 28, 684–703. <https://doi.org/10.1016/j.rbmo.2014.02.004>
- Zhou, X., Liu, F., & Zhai, S. (2007). Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: A systematic review. *Asia Pacific Journal of Clinical Nutrition*, 16(Suppl 1), 383–390.
- Zini, A., & Dohle, G. (2011). Are varicoceles associated with increased deoxyribonucleic acid fragmentation? *Fertility and Sterility*, 96(6), 1283–1287. <https://doi.org/10.1016/j.fertnstert.2011.10.016>

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