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Body mass index and age correlate with antioxidant supplementation effects on sperm quality: Post hoc analyses from a double-blind placebo-controlled trial

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Abstract

Spermatozoa are vulnerable to lack of energy and oxidative stress as a result of elevated levels of reactive oxygen species. Therefore, it is essential that appropriate nutrients are available during maturation. This randomised, double-blind, placebo-controlled trial investigated the effect of 6-month supplementation with carnitines and other micronutrients on sperm quality in 104 subjects with oligo- and/or astheno- and/or teratozoospermia with or without varicocele. Semen analyses were done at the beginning and end of the treatment. In addition to main analyses, post hoc analyses for age and body mass index (BMI) were carried out. Results were interpreted by dividing the population into two age and BMI classes. In 94 patients who completed the study, all sperm parameters increased in supplemented patients compared to the placebo group. A significant (p = .0272) difference in supplementation efficacy was observed for total motility on patients with varicocele and BMI < 25. In the same group, also the progressive motility was significantly superior (p = .0159). For Responder analysis, total motility results were confirmed in both the cited group (p = .0066) and in the varicocele group with BMI < 25 and age < 35 (p = .0078). This study suggests that supplementation is more effective in subjects with varicocele younger than 35 years with BMI < 25.

KEYWORDS

ageing, body mass index (BMI), compounds, male infertility, varicocele

1 | INTRODUCTION

The decline of male fertility is an emerging problem that has been widely reported. A recent meta-analysis analysing sperm counts in men seeking care for fertility problems in developed countries during the period from 1973 to 2011 showed that sperm counts have decreased by 50%–60% (Levine et al., 2017). Causes for these changes are lifestyle factors and global changes in our eating habits with increasing evidence of obesity, which is associated with infertility and several medical conditions (Porreca et al., 2018; Rufus, James, & Michael, 2018). While an association between obesity and female infertility has been clearly proven

(Pinborg et al., 2011), a definitive association with male infertility is still lacking. Nevertheless, a relationship can be postulated when examining the decline in male fertility and the concomitant increase in obesity (Verón et al., 2018). The potential relationship between male infertility and obesity could be due to a decrease in androgen secretion resulting in poorer semen quality and a deterioration in mitochondrial activity (Rufus et al., 2018). Male age could be another factor negatively influencing male fertility. In general, men maintain a certain lifelong fertility, but hormonal status, sexual function and sperm production can decline with advanced paternal age (Belloc et al., 2014). Significant decreases in semen volume, motility, morphology and sperm count have also been reported in men with advanced age (Hellstrom et al., 2006; Kidd, Eskenazi, & Wyrobek, 2001). Moreover, sperm chromatin and DNA integrity have been shown to be negatively impacted with ageing and obesity (Das et al., 2013; Thomsen, Humaidan, Bungum, & Bungum, 2014).

As another major cause of male infertility, oxidative stress (OS) may affect the male germ cells, causing sperm DNA damage and dysfunction. As male germ cells have an extraordinary high content of polyunsaturated fatty acids in plasma membranes, spermatozoa are highly vulnerable to reactive oxygen species (ROS), which in turn leads to membrane damage through lipid peroxidation, and finally, cell death is caused by functional alterations (Agarwal et al., 2014). OS occurs when there is an imbalance between oxidants and antioxidants in favour of the oxidants. For normal sperm function, including chromatin compaction in maturing spermatozoa during epididymal transit, a delicate balance of reduction and oxidation is required (Agarwal, Hamada, & Esteves, 2012; Wright, Milne, & Leeson, 2014). Elevated ROS levels without adequate compensation damage fertility, but a small quantity of ROS is required to trigger essential physiological reactions for normal sperm maturation and function (Agarwal et al., 2014). Ageing is also related to an increase in lipid peroxidation, oxidation of proteins and DNA (Harman, 1992). Since obesity is associated with a general systemic inflammatory status, this also results in OS. More specifically, leptin, interleukin cascades, TNF-α accumulation and other mechanisms are able to produce large amounts of peroxynitrite and other oxidants (Abdali, Samson, & Grover, 2015).

Antioxidants including vitamins, metabolic coenzymes, carnitines (L-carnitine and acetyl-L-carnitine) and micronutrients (zinc, selenium, folic acid) are required for sperm formation and maturation and, due to poor diet, are often deficient, resulting in a decrease in the antioxidant status as well as mitochondrial dysfunction (Busetto et al., 2018; WHO, 2011). Several studies demonstrate that antioxidant supplementation could positively act on fertility by improving sperm quality and is therefore recommended as potentially effective therapy for the treatment of male infertility. With ageing and obesity as causes of OS, a therapeutic strategy could be to use these supplements to minimise free radical damage, increase sperm energy metabolism and thereby improve cellular processes connected with the formation and maturation of spermatozoa. In particular, it is known that antioxidants are able to protect spermatozoa from ROS damages, decrease DNA fragmentation, reduce cryodamage to spermatozoa and block premature sperm maturation (Busetto et al., 2018).

If ageing is increasingly connected with failure to achieve fatherhood and obesity showing growing evidence of being negatively associated with male fertility, these variables should correlate with male infertility. At present, no studies investigated the correlation of age and body mass index (BMI) with the efficiency of a medical therapy in infertile men. Therefore, the objective of this randomised, double-blind, placebo-controlled trial was to evaluate the effect of antioxidant supplementation with selected natural compounds on sperm quality. The effects were evaluated in subjects with oligo- and/or astheno- and/or teratozoospermia and were subsequently analysed across different age and BMI classes.

2 | MATERIALS AND METHODS

2.1 | Patients and study design

The present analysis stems from the extensive database that has been created to determine the effects of antioxidant supplementation on semen quality. This database includes 104 infertile patients with oligo- and/or astheno- and/or teratozoospermia with an average age of 32.5 years (range 18–48), enrolled in a randomised, double-blind, placebo-controlled trial between December 2014 and June 2015. All participants were enrolled at the Andrology clinic at the Department of Gynecological-Obstetric Sciences and Urological Sciences, "Sapienza" Rome University. Included are 52 patients with grade I–III varicoceles and 52 patients without varicocele that were divided into supplementation or placebo groups (Busetto et al., 2018).

The Ethics 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. Informed consent was documented for every patient who participated in the study.

The study was registered on clinicaltrial.gov, and the assigned number is NCT04177667.

2.2 | Randomisation and blindness

The randomisation list was prepared with the nQuery Advisor nTerim 2.0 (2012) program. Two separate lists, one for each varicocele stratum, were prepared. Subjects were randomly assigned to one of the two regimens receiving either the supplementary active product or the placebo product.

TABLE 1 Sperm concentration (10^6 ml)

Group	Class	Statistics	Placebo	Supplementation	*p*-value
With varicocele	Age < 35	*N*	13	18	.6365
		Mean	5.27	9.02	
		Std. Dev.	21.03	21.92	
Without varicocele	Age < 35	*N*	18	16	.2372
		Mean	6.79	14.76	
		Std. Dev.	20.76	17.37	
With varicocele	Age ≥ 35	*N*	13	8	.1296
		Mean	−4.98	8.43	
		Std. Dev.	21.22	13.79	
Without varicocele	Age ≥ 35	*N*	8	10	.6744
		Mean	−5.66	−2.55	
		Std. Dev.	14.56	15.91	
With varicocele	BMI < 25	*N*	19	20	.1173
		Mean	0.89	11.57	
		Std. Dev.	20.06	21.46	
Without varicocele	BMI < 25	*N*	22	21	.7702
		Mean	4.16	5.9	
		Std. Dev.	20.32	18.46	
With varicocele	BMI ≥ 25	*N*	7	6	.8867
		Mean	−1.87	−0.28	
		Std. Dev.	26.18	4.48	
Without varicocele	BMI ≥ 25	*N*	4	5	.1138
		Mean	−3.63	17.34	
		Std. Dev.	16.22	18.07	
With varicocele	Age < 35 & BMI < 25	*N*	10	13	.3749
		Mean	4.95	13.15	
		Std. Dev.	16.28	24.72	
Without varicocele	Age < 35 & BMI < 25	*N*	15	13	.4256
		Mean	7.32	13.48	
		Std. Dev.	22.74	16.45	

This is a double-blind study, and neither the patient nor investigators, responsible for collecting data or analysing laboratory specimens, were knowledgeable regarding the assignment of active or placebo product. A file was maintained at each of the sites under the responsibility of the primary investigator which provided the product identification for each subject. Once the subject entered into the study, they were assigned a unique study identification number.

2.3 | Inclusion and exclusion criteria

In our trial, we have included men aged between 18 and 50 years, with oligo- and/or astheno- and/or teratozoospermia, with or without varicocele (not surgically treated) and men from couples with history of difficulty conceiving for more than 12 months. Patients without varicocele were affected by idiopathic male infertility. A complete check-up to exclude any other cause of infertility (history, examination, complete ultrasound and Doppler, hormones and genetic tests) was done to all participating subjects. Even female partners have been studied, and it was required: regular menstrual cycle, age < 40 and couples not undergoing any assisted reproductive technology (ART).

Exclusion criteria were as follows: known hypersensitivity to any of the compound, 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, excessive consumption of alcohol or regular use of illicit or 'recreational' drugs, positive serology for HIV, subjects following any special diet or taking antioxidants, any systemic condition which in the opinion of the investigator might put the subject at risk by participation in this study and subjects involved in any other clinical trials.

TABLE 2 Volume of ejaculate (ml)

Group	Class	Statistics	Placebo	Supplementation	*p*-value
With varicocele	Age < 35	*N*	13	18	.2134
		Mean	−0.35	0.28	
		Std. Dev.	1.03	1.58	
Without varicocele	Age < 35	*N*	18	16	.4411
		Mean	−0.14	−0.5	
		Std. Dev.	1.42	1.21	
With varicocele	Age ≥ 35	*N*	13	8	.5568
		Mean	−0.26	0.09	
		Std. Dev.	1.22	1.33	
Without varicocele	Age ≥ 35	*N*	8	10	.6719
		Mean	0.66	0.46	
		Std. Dev.	0.89	1.06	
With varicocele	BMI < 25	*N*	19	20	.3345
		Mean	−0.25	0.16	
		Std. Dev.	1.22	1.4	
Without varicocele	BMI < 25	*N*	22	21	.929
		Mean	0.13	0.17	
		Std. Dev.	1.41	1.12	
With varicocele	BMI ≥ 25	*N*	7	6	.2864
		Mean	−0.46	0.43	
		Std. Dev.	0.95	1.85	
Without varicocele	BMI ≥ 25	*N*	4	5	.0514
		Mean	−0.05	−1.38	
		Std. Dev.	0.79	0.89	
With varicocele	Age < 35 & BMI < 25	*N*	10	13	.3141
		Mean	−0.54	0.05	
		Std. Dev.	1.09	1.55	
Without varicocele	Age < 35 & BMI < 25	*N*	15	13	.864
		Mean	−0.13	−0.22	
		Std. Dev.	1.54	1.1	

2.4 | Treatment

In accordance with the randomisation schedule, subjects received 2 packets of either supplement (1,000 mg of L-carnitine, 725 mg of fumarate, 500 mg of acetyl-L-carnitine, 1,000 mg of fructose, 50 mg of citric acid, 50 µg of selenium, 20 mg of coenzyme Q10, 90 mg of vitamin C, 10 mg of zinc, 200 µg of folic acid and 1.5 µg of vitamin B12—Alfasigma Health Science, Utrecht, The Netherlands) or placebo daily for 6 months. Semen parameters were evaluated in a standard semen analysis at the beginning of the treatment (V1) and after completing 6 months of therapy (V2). Variables taken into consideration were ejaculate volume (ml), total sperm count (10^6), progressive motility (%), total motility (%) and normal sperm morphology (%). Pregnancy rate was included as a secondary outcome. An internal quality control, including intra-operator and/or inter-operator controls, was performed. An external quality control, as well, was included, in order to provide a blind evaluation of semen samples regarding concentration, motility and morphology.

2.5 | Statistical Analyses

Analysis of covariance was performed with 2 groups (verum or placebo, with and without varicocele) and was defined as $f = \sigma m/\sigma = 0.25$ with the correlation coefficient (R^2) between the baseline and final equal to 0.50. It was also designated that $\alpha = 0.05$ (significance) and $\beta = 0.20$ (power of 80%). Up to 15% of patients dropping out of the study were estimated, and 104 patients (52 per arm) were enrolled.

All continuous variables are reported as mean, median, standard deviations, and minimum and maximum values. Discrete and nominal variables are reported as frequency and percentage in contingency tables. The basal homogeneity of the groups was tested using analysis of variance (ANOVA) with two levels (drug and varicocele). 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

TABLE 3 Total sperm count (10^6)

Group	Class	Statistics	Placebo	Supplementation	*p*-value
With varicocele	Age < 35	*N*	13	18	.149
		Mean	12.1	50.27	
		Std. Dev.	72.77	69.26	
Without varicocele	Age < 35	*N*	18	16	.316
		Mean	28.9	41.6	
		Std. Dev.	31.54	41.02	
With varicocele	Age ≥ 35	*N*	13	8	.1219
		Mean	−12.13	42.78	
		Std. Dev.	59.59	96.79	
Without varicocele	Age ≥ 35	*N*	8	10	0.812
		Mean	14.86	19.55	
		Std. Dev.	37.44	43.29	
With varicocele	BMI < 25	*N*	19	20	.0272
		Mean	0.4	55.63	
		Std. Dev.	65.34	83.1	
Without varicocele	BMI < 25	*N*	22	21	.6528
		Mean	30.6	35.71	
		Std. Dev.	30.34	42.81	
With varicocele	BMI ≥ 25	*N*	7	6	.5185
		Mean	−1.15	22.39	
		Std. Dev.	74.28	47.17	
Without varicocele	BMI ≥ 25	*N*	4	5	.2853
		Mean	−8.55	22.22	
		Std. Dev.	33.19	43.88	
With varicocele	Age < 35 & BMI < 25	*N*	10	13	.0789
		Mean	1.88	58.25	
		Std. Dev.	68	75.81	
Without varicocele	Age < 35 & BMI < 25	*N*	15	13	.4737
		Mean	33.87	43.49	
		Std. Dev.	30.79	39.14	

Wilcoxon signed-rank test was used in the comparisons before/after by group.

In addition to the main analyses, as designed in the study protocol, the present post hoc analyses were carried out on the samples as categorised by age/BMI and presence/absence of varicocele. Eight subgroups were considered (two age classes and two weight classes and presence/absence of varicocele): age less (1) than or greater (2) than 35 years old and BMI less (a) than or greater (b) than 25. The latter cut-off was suggested by the clinicians since that level is usually considered as the upper limit of the range for the normal BMI class while the age cut-off, that is 35 years, was adopted as in our opinion is considered, in western countries, as the common age for seeking paternity. Age was calculated in years, and BMI was calculated by the standard method of kg/m^2. All the main semen parameters were analysed as difference at the final visit from the baseline (values before the trial). The *t* test was adopted for detecting possible differences between the two treatment groups. BMI and age were also analysed in combination, to obtain another evaluation and to see whether the two factors further changed therapy effect. Once the classes in which the supplementary product seemed to be more effective were identified, the final analysis was performed conducting the same test. In order to confirm the results, the data were arranged as a before/after dataset and the patients were categorised as Responder/Nonresponder (i.e. a patient was considered as 'Responder' if he improved the results at final visit from baseline). The chi-square test was carried out on those data. SAS® version 9.4 (Cary, NC, USA) was used for performing all statistical analyses.

3 | RESULTS

Out of 104 enrolled patients, 94 completed the study (90.4%), with the homogeneity tests showing two well-balanced groups. Adverse events occurred only in the supplement group, and all events were not serious, with four patients experiencing nausea and three having vertigo or headache.

TABLE 4 Total sperm count (10^6)—chi-square test

Group	Treatment	Nonresponder	Responder	Total	*p*-value
Age < 35 With varicocele	Supplementation	5 (27.8%)	13 (72.2%)	18	.0601
	Placebo	8 (61.5%)	5 (38.5%)	13	
	Total	13	18	31	
Age < 35 Without varicocele	Supplementation	2 (12.5%)	14 (87.5%)	16	.2715
	Placebo	5 (27.8%)	13 (72.2%)	18	
	Total	7	27	34	
BMI < 25 With varicocele	Supplementation	5 (25.0%)	15 (75.0%)	20	.0066
	Placebo	13 (68.4%)	6 (31.6%)	19	
	Total	18	21	39	
BMI < 25 Without varicocele	Supplementation	4 (19.0%)	17 (81.0%)	21	.7669
	Placebo	5 (22.7%)	17 (77.3%)	22	
	Total	9	34	43	
Age < 35 and BMI < 25 With varicocele	Supplementation	2 (15.4%)	11 (84.6%)	13	.0078
	Placebo	7 (70.0%)	3 (30.0%)	10	
	Total	9	14	23	
Age < 35 and BMI < 25 Without varicocele	Supplementation	1 (7.7%)	12 (92.3%)	13	.3533
	Placebo	3 (20.0%)	12 (80.0%)	15	
	Total	4	24	28	

3.1 | Semen volume

Overall, results showed no difference in semen volume, before and after the treatment, both in the placebo group (p = .6787) and the supplemented group (p = .6271). Including only varicocele patients, before and after treatment, no difference was observed in both the placebo and supplemented group, p = .2250 and p = .3632 respectively. Patients without varicocele showed no statistically significant difference before/after in both the placebo group (p = .7711) and the supplemented group (p = .8753).

3.2 | Total sperm count

Comparing all patients of the placebo group, the treatment resulted in no change for the total sperm count (p = .2030). In contrast, total sperm count in the supplemented group increased highly significantly (p < .0001). For patients suffering from varicocele, no difference was observed in the placebo group (p = .8764), while a statistically significant (p = .0066) improvement in favour of the supplemented group was obvious. For nonvaricocele subjects, in both the placebo and supplementation groups, no significant difference was observed (p = .4259 and p = .2460).

3.3 | Progressive motility

For progressive motility, the treatment resulted in no difference (p = .1567) in the placebo group. In contrast, in the supplemented group a significant (p = .0012) increase in progressive motility was obvious at the final visit. In varicocele patients, no difference was seen with the placebo (p = .1570), whereas a significant difference was recorded in the supplemented group. In nonvaricocele patients, the results were again significant in the treated arm (p = .0311) and not significant in the placebo arm (p = .4866).

3.4 | Total motility

The overall results for total motility, before and after therapy, revealed no difference in the placebo group (p = .1483), but a significant difference (p < .0001) in the supplemented group. In the varicocele group, the results in the placebo group were once again statistically not significant (p = .1214), while total motility in the treated group was significantly (p = .0065) higher. As for the group without varicocele, the results did not differ (p = .5604) in the placebo group when comparing the initial results with those at the final visit. In contrast, in the treated group, total motility was significantly (p = .0028) higher.

3.5 | Sperm morphology

Results reported no difference in normal sperm morphology between the placebo group and the supplemented group, at baseline (p = .2062) and at the end of the study (p = .3791). On the other hand, whereas the placebo group had significantly lower values of normal sperm morphology at the final visit (p = .0105), results in the supplemented group did not differ (p = .1310).

TABLE 5 Progressive motility (%)

Group	Class	Statistics	Placebo	Supplementation	*p*-value
With varicocele	Age < 35	*N*	13	18	.5159
		Mean	2.16	4.18	
		Std. Dev.	7.1	9.28	
Without varicocele	Age < 35	*N*	18	16	.3979
		Mean	4.11	7.71	
		Std. Dev.	9.35	14.84	
With varicocele	Age ≥ 35	*N*	13	8	.3628
		Mean	1.58	4.1	
		Std. Dev.	6.28	5.54	
Without varicocele	Age ≥ 35	*N*	8	10	.0831
		Mean	−5.15	3.52	
		Std. Dev.	11.14	8.79	
With varicocele	BMI < 25	*N*	19	20	.0159
		Mean	0.87	5.93	
		Std. Dev.	4.48	7.55	
Without varicocele	BMI < 25	*N*	22	21	.1905
		Mean	1.85	6.69	
		Std. Dev.	9.67	13.89	
With varicocele	BMI ≥ 25	*N*	7	6	.2524
		Mean	4.57	−1.75	
		Std. Dev.	10.4	8.06	
Without varicocele	BMI ≥ 25	*N*	4	5	.5133
		Mean	−1.95	3.64	
		Std. Dev.	16.5	7.22	
With varicocele	Age < 35 & BMI < 25	*N*	10	13	.0599
		Mean	−0.12	6.12	
		Std. Dev.	4.5	9.07	
Without varicocele	Age < 35 & BMI < 25	*N*	15	13	.3311
		Mean	4.12	8.94	
		Std. Dev.	8.88	16.28	

3.6 | Pregnancy rate

As a secondary outcome, 12 pregnancies occurred during the 6 months of follow-up time: 10 in the supplementation group and 2 in the placebo group. The chi-squared test showed that the difference between the two groups was statistically significant ($p = .0141$).

3.7 | Body mass index and age correlation

One of the primary aims of this study was to correlate the results of the semen analysis with BMI and age. In particular, we wanted to see if ageing and obesity status would decrease efficacy of the supplementary antioxidant treatment on main sperm parameters (see Tables 1–7). For BMI, a significant difference was observed in the BMI < 25 group with varicocele for total sperm count ($p = .0272$, see Table 3) and progressive motility ($p = .0159$, see Table 5). No statistical significance was observed in the combined classes. The results were partially confirmed by carrying out the chi-square test on the data arranged as 'Responder/Nonresponder' (see above). As for the total sperm count, in both the BMI < 25 and the combined varicocele group (i.e. BMI < 25 and age < 35) a statistical difference was observed ($p = .0066$ and $p = .0078$, respectively, see Table 4). These post hoc analyses suggest that the nutritional supplement seems to be more effective in subjects younger than 35 years with a BMI below 25.

4 | DISCUSSION

Ageing and obesity are two modern global problems. BMI is the common parameter used to measure obesity, and a value ≥ 25 is considered overweight (WHO, 2011). Obesity harms health and longevity; increased visceral fat tissue adds to the risk of age-related disease

TABLE 6 Progressive motility (%)—chi-square test

Group	Treatment	Nonresponder	Responder	Total	*p*-value
Age < 35 With varicocele	Supplementation	8 (44.4%)	10 (55.6%)	18	.3473
	Placebo	8 (61.5%)	5 (38.5%)	13	
	Total	16	15	31	
Age < 35 Without varicocele	Supplementation	4 (25.0%)	12 (75.0%)	16	.3876
	Placebo	7 (38.9%)	11 (61.1%)	18	
	Total	11	23	34	
BMI < 25 With varicocele	Supplementation	5 (25.0%)	15 (75.5%)	20	.0368
	Placebo	11 (57.9%)	8 (42.11%)	19	
	Total	16	23	39	
BMI < 25 Without varicocele	Supplementation	7 (33.3%)	14 (66.7%)	21	.2681
	Placebo	11 (50.0%)	11 (50.0%)	22	
	Total	18	25	43	
Age < 35 and BMI < 25 With varicocele	Supplementation	4 (30.8%)	9 (69.2%)	13	.0619
	Placebo	7 (70.0%)	3 30.0%)	10	
	Total	11	12	23	
Age < 35 and BMI < 25 Without varicocele	Supplementation	3 (23.1%)	10 (76.9%)	13	.3389
	Placebo	6 (40.0%)	9 (60.0%)	15	
	Total	9	19	28	

and early mortality, in part because of a systemic state of increased oxidative stress and inflammation (Tzanetakou, Katsilambros, Benetos, Mikhailidis, & Perrea, 2012). Ageing is connected with cellular ageing and endocrinological and metabolic changes. A cells' age is based on the number of times they have replicated. However, due to ageing, telomeres are shortened after many replications with the result that the genetic material is no longer able to be copied accurately. Even hormone levels fluctuate through life and are thus able to drive cells' behaviour. Exposure to toxins, sun, foods, pollution and smoking leads to tissue damage as the body falls behind in its ability to maintain and repair cells, tissues and organs. Even metabolic processes and cellular energy production are negatively affected over time (Labat-Robert & Robert, 2015; Russell & Kahn, 2007). Thus, ageing and obesity increase the onset of metabolic imbalances, leading to a reduced lifespan and accelerated cellular degradation processes such as deterioration of the structure and function of organs associated with genetic instability and disturbance of homeostatic pathways (Ahima, 2009). Male fertility, as an important biological process, may suffer as a result of increased BMI and age. Nevertheless, mechanisms that directly link obesity and ageing with lower fertility have not been confirmed.

Kasturi et al. linked obesity to metabolic syndrome (MetS), and the accompanying pro-inflammatory state may lead to inflammation and oxidative stress, which can in turn cause DNA alterations and testicular damage (Kasturi, Tannir, & Brannigan, 2008). Other authors confirmed this relationship by analysing sperm parameters and body weight. Even sperm DNA integrity worsens with increased body weight as the overweight status is directly associated with a higher DNA fragmentation index (DFI) (Dupont et al., 2013; Kort et al., 2006). Another important mechanism relating infertility with an increased BMI is found in hormonal changes related to obesity. Decreased sex hormone-binding globulin (SHBG) binding capacity, increased estrogens, decreased luteinising hormone (LH) and decreased testosterone are mainly caused by hyperinsulinemia and hyperlipidemia and are common in obese patients (Aggerholm, Thulstrup, Toft, Ramlau-Hansen, & Bonde, 2008). All of these hormonal alterations can affect sperm parameters by acting on mitochondrial function, DNA fragmentation, oxidative stress and increased likelihood of miscarriage (Engin-Ustun et al., 2018). On the other hand, some authors reported that an association between BMI and male fertility cannot be established and patients should be reassured that semen quality is not affected by obesity (Rufus et al., 2018). Even some meta-analyses are contradictory, reporting in one case a statistically significant association between body weight and semen parameters, while in other studies no relation was found (MacDonald, Herbison, Showell, & Farquhar, 2010; Sermondade et al., 2013).

Paternal age is reported to be another important factor involved in fertility changes (Mazur & Lipshultz, 2018). As with the overweight status, hormones are altered and FSH and testosterone decreases are commonly observed in older men. Reportedly, an FSH decrease is the cause of reduced Sertoli cell function, germ cell degeneration and reduced daily sperm production (Johnson, Grumbles, Bagheri, & Petty, 1990). Moreover, sperm and seminal parameters are also affected by ageing with declining semen volume, sperm motility and morphology (Belloc et al., 2014; Sloter et al., 2006). DNA fragmentation and sperm chromatin compaction are altered and associated with lower sperm quality and reduced pregnancy rate (Loft et al., 2003). Several trials correlating age with DNA quality have shown

TABLE 7 Total motility (%)

Group	Class	Statistics	Placebo	Supplementation	*p*-value
With varicocele	Age < 35	*N*	13	18	.3438
		Mean	1.89	5.06	
		Std. Dev.	7.65	9.91	
Without varicocele	Age < 35	*N*	18	16	.0711
		Mean	−0.5	7.53	
		Std. Dev.	12.21	12.84	
With varicocele	Age ≥ 35	*N*	13	8	.8897
		Mean	3.93	4.53	
		Std. Dev.	10.26	7.72	
Without varicocele	Age ≥ 35	*N*	8	10	.2955
		Mean	1.38	8.14	
		Std. Dev.	13.87	12.63	
With varicocele	BMI < 25	*N*	19	20	.1766
		Mean	1.81	5.73	
		Std. Dev.	8.27	9.44	
Without varicocele	BMI < 25	*N*	22	21	.1127
		Mean	2.45	8.4	
		Std. Dev.	10.25	13.64	
With varicocele	BMI ≥ 25	*N*	7	6	.4911
		Mean	5.91	2.12	
		Std. Dev.	10.61	8.18	
Without varicocele	BMI ≥ 25	*N*	4	5	.0624
		Mean	−12.98	5.1	
		Std. Dev.	17.22	6.05	
With varicocele	Age < 35 & BMI < 25	*N*	10	13	.0904
		Mean	0.28	7.01	
		Std. Dev.	6.77	10.38	
Without varicocele	Age < 35 & BMI < 25	*N*	15	13	.1948
		Mean	1.46	7.53	
		Std. Dev.	9.95	14.09	

that DNA damage is commonly present in ageing men and this has been observed not only in infertile populations, but also even in healthy people (Belloc et al., 2009; Heidari et al., 2016). Isolated sperm DNA defects are also more prevalent in older men as compared to younger men [8].

With regard to varicocele, there are numerous reports associating this condition with male infertility (Agarwal et al., 2012; Busetto et al., 2018; Heidari et al., 2016). This condition is characterised by elevated seminal ROS levels and oxidative stress leading to sperm dysfunction. Mitochondria, when damaged or dysfunctional, are a key source of ROS, and their gene alterations can affect the respiratory electron transfer chain (Heidari et al., 2016). This process, maybe because of ATP synthesis alteration, ultimately interferes with sperm motility and fertility (Heidari et al., 2016). Latest reports in the field of proteomics show that the proteomic profile of patients with varicoceles are significantly different (Panner & Agarwal, 2019).

Antioxidants are commonly used as medical therapy for male infertility, and studies demonstrate that their use has a beneficial effect on fertility (Wright et al., 2014; Busetto et al., 2018). Even if low levels of reactive oxygen species are required for sperm production, oxidative stress represents the main cause of DNA fragmentation and sperm dysfunction (Aitken, 1997). In fact, when OS is high and an uncontrolled release of ROS can be found, sperm motility is low, while membrane lipid peroxidation, protein oxidation and sperm DNA damage are elevated (de Lamirande, Jiang, Zini, Kodama, & Gagnon, 1997). In such cases, antioxidants are recommended as potentially effective therapy for the treatment of male infertility as these compounds scavenge excessive ROS. In turn, this can then result in increased sperm quality and decreased DNA fragmentation through reduced oxidative stress (Micic et al., 2019). Supplementation of vital antioxidants in the reproductive tract in antioxidant-deficient patients could be a strategy to increase the ROS-scavenging capacity of seminal plasma and thereby reducing seminal OS. On the other

hand, it is important to avoid complete ROS clearance because of their essential role in sperm maturation and function (de Lamirande et al., 1997; Zini, San Gabriel, & Baazeem, 2009). Otherwise, the body might go from oxidative stress into a condition characterised by an excessive amount of antioxidants, namely reductive stress, which is reportedly as dangerous as oxidative stress and is involved in numerous pathologies including cardiomyopathy, cancer, Alzheimer's disease or embryogenesis defects (Henkel, Sandhu, & Agarwal, 2019; Lloret, Fuchsberger, Giraldo, & Vina, 2016; Rajasekaran et al., 2007).

Even though the association of age and BMI as potential risk factors for male infertility has not yet been definitively established, we investigated the effect of ageing and obesity on the efficacy of an antioxidant supplementation therapy for male fertility. To the best of our knowledge, this report is the first analysing the effect of antioxidants on sperm parameters with particular emphasis on how such therapy could be affected by age and BMI. The results are surprising because initially we expected a strong beneficial effect of the supplementation in older and obese patients because of the oxidative stress typical of these conditions. In reality, we found exactly the opposite and our data appear to show that in patients older than 35 years and in obese men with a BMI higher than 25, the antioxidant treatment was less effective improving semen quality. In particular, we observed a statistically significant difference on the effect of antioxidants on sperm total motility in favour of those younger than 35 and a significant difference on total sperm count, progressive motility and total motility in favour of patients with a BMI less than 25. As a result, we consider supplementation more effective in younger and normal weight subjects. Perhaps, it might be beneficial to increase the antioxidant dosage for obese and older patients in order to increase the efficacy and improve sperm quality. Our results also call for the inclusion of a diagnostic measure of oxidative stress such as oxidation-reduction potential (ORP) in order to establish the need of these patients to be supplemented with antioxidants and treat male infertility (Agarwal & Wang, 2017). Such diagnostic determination of the ORP could assist in better defining the dosage and duration of supplementation therapy and subject of future study.

Despite the significant positive findings, our trial has some limitations. Even as a double-blind placebo-controlled study with a balanced population and very strict inclusion and exclusion criteria, we did not include sperm DNA fragmentation as an important parameter to evaluate infertility. Also, an oxidative stress measure such as ORP was not included. There also may be other factors besides ageing and obesity involved, including lifestyle habits, associated disease and fat distribution, and these may require further analysis.

5 | CONCLUSION

In addition to earlier findings regarding improved sperm parameters in supplemented patients, these post hoc analyses suggest that antioxidant supplementation seems to be more effective on improving sperm parameters in subjects aged less than 35 years old and with BMI below 25.

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