Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia

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Objective: To evaluate the effectiveness of L-carnitine (LC) or L-acetyl-carnitine (LAC) or combined LC and LAC treatment in improving semen kinetic parameters and the total oxyradical scavenging capacity in semen.
Design: Placebo-controlled, double-blind, randomized trial.
Setting: Andrology unit, Department of Internal Medicine, Polytechnic University of Marche, Italy.
Patient(s): Sixty infertile men, ages 20 to 40 years, with the following baseline sperm selection criteria: concentration $>20 \times 10^6$/mL, sperm forward motility <50%, and normal sperm morphology >30%; 59 patients completed the study.
Intervention(s): Patients underwent a double-blind therapy of LC 3 g/d, LAC 3 g/d, a combination of LC 2 g/d and LAC 1 g/d, or placebo. The study design was 1 month of run in, 6 months of therapy or placebo, and 3 months of follow-up evaluation.
Main Outcome Measure(s): Variations in semen parameters used for patient selection, and variations in total oxyradical scavenging capacity of the seminal fluid.
Result(s): Sperm cell motility (total and forward, including kinetic features determined by computer-assisted sperm analysis) increased in patients to whom LAC was administered both alone or in combination with LC; combined LC + LAC therapy led to a significant improvement of straight progressive velocity after 3 months. The total oxyradical scavenging capacity of the semen toward hydroxyl and peroxyl radicals also increased and was positively correlated with the improvement of kinetic features. Patients with lower baseline values of motility and total oxyradical scavenging capacity of the seminal fluid had a significantly higher probability of responding to the treatment.
Conclusion(s): The administration of LC and LAC is effective in increasing sperm kinetic features in patients affected by idiopathic asthenozoospermia and improves the total oxyradical scavenging capacity of the seminal fluid in the same population. (Fertil Steril® 2005;84:662–71. ©2005 by American Society for Reproductive Medicine.)

Key Words: Male infertility, carnitine therapy, asthenozoospermia, total scavenging capacity

L-carnitine (LC) plays a central role in cellular energetic metabolism, acting as a shuttle of the activated long-chain fatty acids (acyl-CoA) into the mitochondria, where beta-oxidation take place (1–3). An important role in sperm cell metabolism is strongly suggested by the high levels of LC found in epididymal fluid due to an active secretory mechanism (4), and there is also evidence that the initiation of sperm motility is related to an increase of LC in the epididymal lumen and L-acetyl-carnitine (LAC) in sperm cells (5–7).

An excess of reactive oxygen species (ROS) and other oxidant radicals has been associated with male infertility (8–15). The total oxyradical scavenging capacity (TOSC) is a recently developed assay measuring the overall capability of biological fluids or cellular antioxidants to neutralize the toxicity of various oxyradicals (16, 17). The TOSC assay can discriminate between different forms of ROS, allowing identification of the role of specific antioxidants and/or their pathway of formation in the onset of toxicologic or pathologic processes.

Previous application of the TOSC assay in andrology led us to show a reduced antioxidant efficiency in seminal fluid of infertile men with a significant correlation between the scavenging capacity toward hydroxyl radicals and parame-

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ters of sperm cell motility (18). Furthermore, there is ample evidence that the treatment of asthenozoospermic infertile men with antioxidants leads to improvements in semen quality parameters (19, 20) and pregnancy rate (20).

L-carnitine has been used as a therapeutic approach in selected forms of oligoasthenoteratozoospermia (21–25), based on its role on energetic metabolism. Although a secondary role of LC as antioxidant has been suggested (26), the mechanism involved in protection against oxidative damage induced by ROS has yet to be elucidated.

We here report on a 6-month, double-blind, randomized, placebo-controlled trial using LC or LAC or combined LC and LAC treatment in infertile men affected by idiopathic asthenozoospermia. Evaluation of the effectiveness of these treatments in improving semen kinetic parameters and the variation of total oxyradical scavenging capacity in semen after treatment were the end points of the study.

MATERIALS AND METHODS

Patient Selection

Sixty patients (range: 24 to 38 years; mean age: 30 years) affected by idiopathic asthenozoospermia were enrolled in the study. The patients were selected at the Andrology Unit of the Division of Endocrinology, Umberto I Hospital, Polytechnic University of Marche, Ancona, Italy. All of the men had presented a clinical history of primary infertility >2 years, and they underwent medical screening, including history and clinical examination. Testicular volume was evaluated in each patient using Prader's orchidometer. To accomplish a complete diagnosis, the following investigations were also performed: semen analysis; Mar-test (SperMar test, CGA, Florence, Italy) for antispermatozoa antibodies (Ab); sperm culture and urethral specimen collection to detect *Chlamydia* and *Mycoplasma urealyticum*; follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), estradiol (E_2), and prolactin (PRL) assays using commercial radioimmunoassay kits; and testicular, prostatic, and seminal vesicle ultrasonography and echocolor Doppler of venous spermatic plexus to detect anatomic abnormalities and varicocele.

No female-related factors were apparently involved in sterility; all partners (range: 21 to 32 years; mean age: 26 years) ovulated regularly, as formally proven by biphasic basal body temperature and luteal phase progesterone levels. In each case, no anatomic abnormalities were detected on ultrasound evaluation of the partner ovaries and uterus; no abnormal fallopian tube anatomy was detected after hysterosalpingography.

Study Design and Treatments

The selected patients underwent a double-blind therapy of: LC (10-mL phials containing 3 g/d orally of Carnitene [Sigma Tau, Pomezia, Italy], n = 15); or LAC (3 g/d oral tablets, Zibren [Sigma Tau], n = 15); or a combination of LC (10-mL phials containing 2 g/d orally of Carnitene) and LAC (tablets containing 1 g/d orally of Zibren) (n = 15); or a seemingly identical placebo (each 10-mL placebo phial containing: malic acid, sodium benzoate, sodium saccharinate dihydrate, anhydrous sodium citrate, pineapple flavoring, and demineralized water). Each placebo tablet contained a core with 1-hydro lactose, magnesium stearate, polyvinylpyrrolidone, corn starch, and a coating with cellulose acetophtalate, dimethicone, ethylphtalate (Sigma Tau). All patients were given a total of one phial and two tablets three times a day. The carnitine dose chosen was the one most commonly used for treatment of other diseases (e.g., nervous system, heart, muscular diseases), and was similar to that used in other trials on male infertility by other investigators (21–25).

The study design was 1 month of run-in, 6 months of therapy (45 patients) or placebo (15 patients), and further 3 months of follow-up evaluation (controls at months T-1, T0, T+3, T+6, T+9). Monthly evaluation of two semen samples before the beginning of treatment (T-1, T0) was carried out to test semen parameter stability in each patient, as recommended by the World Health Organization (27).

At various time points, the analyses were carried out. Semen analysis was performed at months T-1, T0, T+3, T+6, and T+9, including computer-assisted sperm analysis (CASA) at months T0, T+3, T+6, T+9 to evaluate modifications in semen parameters (27). To evaluate any variations during therapy, TOSC was performed of the seminal fluid toward different reactive oxygen species (ROS) at months T0 and T+6 (16, 17). All analyses were performed on both treated and placebo patients. Patient compliance and possible side effects were also noted, and blood analysis was carried out at T0 and T+6 to check the treatment's safety.

On the basis of the previous results from our group and other investigators (20–25), improvements in sperm motility (both total and forward) were considered the main measurement of efficacy.

The present study was approved by the Italian Ministry of Health and the institutional review board and ethics committee of the faculty of medicine at Ancona University Hospital. All patients provided written informed consent.

Eligibility Criteria

The following criteria were adopted for patient eligibility: [1] age 20 to 40 years, infertility >2 years after regular sexual intercourses with a fertile woman; [2] normal rheologic characteristics (appearance, consistency, and liquefaction) of semen, and volume and pH in the normal range; [3] sperm count $>20 \times 10^6$/mL, sperm motility (forward motility, class a and b, according to World Health Organization criteria) (27) <50%, and normal sperm morphologic features >30%; [4] seminal white blood cells (WBC) $<1 \times 10^6$/mL, negative sperm culture, and *Chlamydia* and *My-*

coplasma urealyticum detection; [5] normal serum levels of gonadotropins, T, E_2, and PRL; [6] absence of infectious genital diseases, anatomic abnormalities of the genital tract including varicocele, and antispermatozoa Ab; [7] absence of systemic diseases or treatment with other drugs within the 3 months before enrollment in the present study; [8] absence of smoking, alcohol, or recreational drug use or of occupational chemical exposure.

Patients were asked to follow a standard diet to avoid affects due to variable carnitine intake in food. None of the patients suffered from carnitine metabolism deficiency.

For inclusion in the trial, patients had to meet the above semen inclusion criteria at both T-1 and T0. This excluded any patient with transient decrease in semen quality during the washout period and those who had any sudden (and independent of treatment) improvement in semen features.

Seminal Fluid Analysis

Semen quality was assessed by the same biologist in terms of sperm concentration, motility, and morphology, in accordance with the World Health Organization criteria (27).

Computer-assisted sperm analysis (CASA) for sperm cells motility assay was performed as previously described elsewhere (20). One semen aliquot (3 μL) was placed in a 20-μm depth chamber. Two 20-μm depth cell–VU chambers (Conception Technologies, La Jolla, CA) were loaded, and six different fields per chamber were randomly examined; at least 200 sperm for each field of the chamber were scored. Movement characteristics were analyzed using an automated analyzer (CellTrack VP110, Motion Analysis Corporation, Palo Alto, CA). Sperm velocity and kinetic characteristics were evaluated only for motile sperm and expressed as mean values considering both curvilinear velocity (VCL) and straight progressive velocity (VSL).

TOSC Assay

Semen samples as 1-mL aliquots were centrifuged after liquefaction at 2,000 rpm for 15 minutes, and supernatant plasma was immediately separated from the pellet of spermatozoa and stored at −80°C until the assay. A small sample of seminal plasma was examined before storage to rule out the presence of spermatozoa in the supernatant.

The TOSC assay is based on the reaction between various forms of ROS and the substrate α-keto-γ-methiolbutyric acid (KMBA) which is oxidized to ethylene. The antioxidant efficiency of a sample is quantified by its ability to scavenge the generated oxyradicals, thus inhibiting their reaction with KMBA and ethylene formation (15). Technical procedures for TOSC assay were performed according to previous reports from our laboratory (18). For the various oxidant-generating systems, TOSC values were quantified using the equation: $\text{TOSC} = 100-(\text{SA}/\text{CA} \times 100)$, where SA and CA are the integrated areas calculated under the least square kinetic curve produced during the reaction course for sample (SA) and control (CA) reactions, respectively.

Statistical Analysis

For each observed variable, descriptive statistics were computed for all treatment groups.

The homogeneity of the four groups of patients before the beginning of treatment (visits T-1 and T0) was evaluated using an analysis of variance with treatment as between factor (four levels: placebo, LC, LAC, combined LC and LAC).

For all the continuous variables measured at visits T-1, T0, T+6, and T+9, the percentage variation with respect to T-1 (or with respect to T0, when most observations at T-1 were missing) was calculated to eliminate the influence of possible differences at the beginning of the study.

To separate the effect of the two molecules LC and LAC, and to evaluate their combination, two new "treatment" variables were computed: the first one, LCTX indicated therapy with LC either alone or in combination with LAC; LACTX indicated administration of LAC either alone or in combination with LC. To assess the significance of time and treatments on the percentage variations, an analysis of variance for repeated measures was performed.

For continuous variables measured at baseline and at the end of treatment, absolute variations were computed, and an analysis of variance with treatments as between factors was performed.

For categorical variables, at each time a χ^2 test was performed to evaluate the association with treatment (taken as one factor at four levels).

For the main parameter of efficacy, sperm motility both total and forward, further analyses were performed. For the two variables, the variations between T0 and T+6 were computed; patients with an improvement greater than or equal to the median of the distribution were defined as "responders." More precisely, two new variables were calculated assuming values 1 and 0, respectively, for responder and nonresponder patients, in terms of both total and forward motility.

To assess whether the baseline values of kinetic and computerized values determined a more consistent variation of sperm motility, two logistic models were computed, considering, respectively, the two responder-variables as dependent, and the baseline values of total motility, VCL, VSL, TOSC, and concentration as independent variables.

RESULTS

One patient dropped out of the study. When the randomization list was opened at the end of the study, 44 out of the 45 patients included in the therapy group (subgroup A: LC, 15 patients; subgroup B: LAC, 15 patients; subgroup C: LC + LAC, 14 patients) and 15 patients included in the placebo group had completed the study.

TABLE 1

Descriptive statistics of sperm variables at each time: mean ± standard deviation.

	Treatment	Month T − 1	Month T0	Month T + 3	Month T + 6	Month T + 9
Sperm total motility						
	Placebo	43.73 ± 10.06	43.93 ± 10.26	44.60 ± 7.68	43.40 ± 9.85	42.73 ± 10.02
	LC	54.33 ± 8.59	51.67 ± 11.08	59.93 ± 8.04	64.53 ± 8.41	54.27 ± 8.96
	LAC	45.07 ± 12.01	43.87 ± 11.36	56.47 ± 11.56	60.43 ± 10.46	50.57 ± 5.71
	LC + LAC	46.73 ± 10.10	44.53 ± 11.84	55.13 ± 10.15	61.07 ± 9.07	49.00 ± 7.80
Sperm forward motility						
	Placebo	24.33 ± 7.93	24.13 ± 7.74	22.33 ± 7.76	24.00 ± 8.50	23.20 ± 8.96
	LC	33.47 ± 6.55	31.20 ± 7.43	38.93 ± 7.09	43.80 ± 7.12	34.00 ± 7.02
	LAC	27.00 ± 10.87	25.53 ± 10.43	34.93 ± 9.24	37.50 ± 9.20	30.21 ± 7.84
	LC + LAC	25.47 ± 8.90	24.60 ± 9.40	33.87 ± 8.37	38.13 ± 8.23	28.47 ± 8.27
Sperm concentration						
	Placebo	35.27 ± 21.98	29.53 ± 10.07	31.40 ± 12.85	33.73 ± 14.36	30.13 ± 9.30
	LC	35.47 ± 9.21	39.00 ± 10.39	41.00 ± 17.34	45.53 ± 21.42	39.40 ± 13.93
	LAC	27.07 ± 6.47	30.40 ± 10.80	39.33 ± 18.05	39.57 ± 19.99	31.21 ± 8.60
	LC + LAC	29.93 ± 10.57	29.40 ± 9.39	36.93 ± 19.71	37.40 ± 16.42	33.27 ± 13.62
Atypical sperm cells						
	Placebo	66.40 ± 6.50	68.20 ± 5.86	67.40 ± 6.42	67.27 ± 6.71	67.53 ± 7.42
	LC	63.13 ± 5.04	62.87 ± 4.69	58.47 ± 6.20	54.87 ± 7.27	58.07 ± 11.82
	LAC	65.93 ± 8.19	67.13 ± 7.06	61.73 ± 6.82	58.93 ± 5.62	60.93 ± 10.12
	LC + LAC	65.40 ± 6.22	67.13 ± 6.01	61.73 ± 5.86	59.60 ± 5.82	61.53 ± 8.84
Semen volume						
	Placebo	2.97 ± 1.36	3.01 ± 0.83	3.08 ± 0.85	2.75 ± 0.68	2.82 ± 0.45
	LC	2.96 ± 0.74	3.12 ± 1.04	3.10 ± 0.68	3.18 ± 0.93	3.03 ± 0.83
	LAC	2.89 ± 0.85	2.59 ± 0.63	2.71 ± 0.62	3.03 ± 0.66	2.76 ± 0.51
	LC + LAC	3.05 ± 0.94	2.87 ± 0.88	2.75 ± 0.80	2.69 ± 0.78	2.50 ± 0.41
Curvilinear velocity						
	Placebo		41.67 ± 14.14	39.67 ± 14.07	42.87 ± 6.83	46.33 ± 11.96
	LC		41.73 ± 13.47	47.73 ± 13.43	57.13 ± 13.95	41.67 ± 6.28
	LAC		39.73 ± 12.57	45.87 ± 11.33	51.79 ± 6.17	44.79 ± 8.19
	LC + LAC		43.00 ± 12.02	44.93 ± 15.72	51.40 ± 13.71	42.53 ± 7.78
Straight progressive velocity						
	Placebo		24.47 ± 15.19	21.80 ± 12.23	15.87 ± 2.47	17.67 ± 2.58
	LC		20.00 ± 7.87	21.13 ± 6.92	21.47 ± 3.52	16.80 ± 2.11
	LAC		23.27 ± 16.28	18.60 ± 5.78	20.36 ± 3.41	16.36 ± 2.41
	LC + LAC		18.60 ± 6.93	25.67 ± 12.75	22.53 ± 10.26	16.73 ± 2.89

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Sperm Output

Table 1 reports mean and standard deviation of sperm variables at each time; the percentage variations with respect to baseline are reported in Table 2. The percentage variations of forward sperm motility in all groups at each time are shown in Figure 1.

The univariate analysis of variance performed on variables (percentage variations compared with T-1 or T0) for the homogeneity at baseline (T0 or T+3) showed that there were no statistically significant differences between groups regarding motility (total and forward), sperm concentration,

TABLE 2

Percentage variations with respect to baseline for sperm variables at each time: mean ± standard deviation.

	Treatment	Month T0	Month T + 3	Month T + 6	Month T + 9
Sperm total motility[a]					
	Placebo	1.30 ± 13.52	4.15 ± 16.26	0.52 ± 17.43	−1.22 ± 17.46
	LC	−5.32 ± 9.91	11.01 ± 8.46	19.90 ± 12.74	0.31 ± 9.65
	LAC	−1.31 ± 23.07	30.83 ± 33.99	41.25 ± 29.98	19.78 ± 28.51
	LC + LAC	−5.16 ± 10.18	19.59 ± 16.63	34.46 ± 26.20	6.75 ± 14.77
Sperm forward motility[a]					
	Placebo	−0.30 ± 9.81	−8.31 ± 13.73	−0.96 ± 18.99	−4.38 ± 21.71
	LC	−5.92 ± 16.21	18.01 ± 15.73	33.08 ± 17.29	2.67 ± 14.40
	LAC	−4.09 ± 27.85	41.85 ± 42.43	56.84 ± 54.23	22.32 ± 29.03
	LC + LAC	−1.86 ± 25.16	43.58 ± 46.86	68.70 ± 78.18	17.73 ± 28.61
Sperm concentration[a]					
	Placebo	−4.31 ± 27.75	−3.17 ± 20.06	6.98 ± 36.97	−1.51 ± 32.69
	LC	11.14 ± 19.58	17.18 ± 45.99	24.50 ± 35.55	12.06 ± 30.16
	LAC	6.95 ± 22.06	46.55 ± 55.80	42.88 ± 50.80	15.90 ± 32.10
	LC + LAC	1.26 ± 19.66	24.34 ± 41.83	29.78 ± 41.53	15.18 ± 42.83
	LC + LAC	1.26 ± 19.66	24.34 ± 41.83	29.78 ± 41.53	15.18 ± 42.83
Atypical sperm cells[a]					
	Placebo	2.92 ± 4.68	1.70 ± 5.97	1.49 ± 6.50	1.86 ± 7.46
	LC	−0.28 ± 4.86	−7.42 ± 6.23	−13.24 ± 7.66	−7.51 ± 19.46
	LAC	2.20 ± 5.48	−6.00 ± 6.90	−10.24 ± 5.56	−6.32 ± 18.07
	LC + LAC	2.90 ± 6.66	−5.27 ± 8.13	−8.35 ± 10.41	−5.36 ± 14.29
Semen volume[a]					
	Placebo	11.76 ± 31.35	19.75 ± 62.98	4.85 ± 40.95	12.03 ± 51.56
	LC	3.63 ± 12.23	6.58 ± 16.51	11.26 ± 34.40	4.91 ± 25.76
	LAC	−5.20 ± 22.97	0.00 ± 27.80	11.63 ± 39.62	2.09 ± 32.97
	LC + LAC	−4.94 ± 17.07	−8.27 ± 16.62	−5.38 ± 38.63	−13.74 ± 19.09
Curvilinear velocity[b]					
	Placebo		2.20 ± 54.71	20.16 ± 67.30	31.24 ± 79.69
	LC		26.00 ± 67.68	64.99 ± 104.59	20.06 ± 74.02
	LAC		30.95 ± 66.09	53.31 ± 71.53	31.23 ± 62.97
	LC + LAC		8.27 ± 33.87	29.05 ± 51.98	8.44 ± 44.17
Straight progressive velocity[b]					
	Placebo		−4.59 ± 37.05	−22.08 ± 27.78	−15.04 ± 25.40
	LC		11.08 ± 22.75	17.07 ± 32.81	−6.45 ± 29.37
	LAC		2.05 ± 43.11	15.57 ± 46.31	−6.78 ± 38.88
	LC + LAC		44.86 ± 76.24	33.26 ± 70.62	−3.15 ± 26.25

[a] Percentage variations with respect to month T-1.
[b] Percentage variations with respect to month T0.

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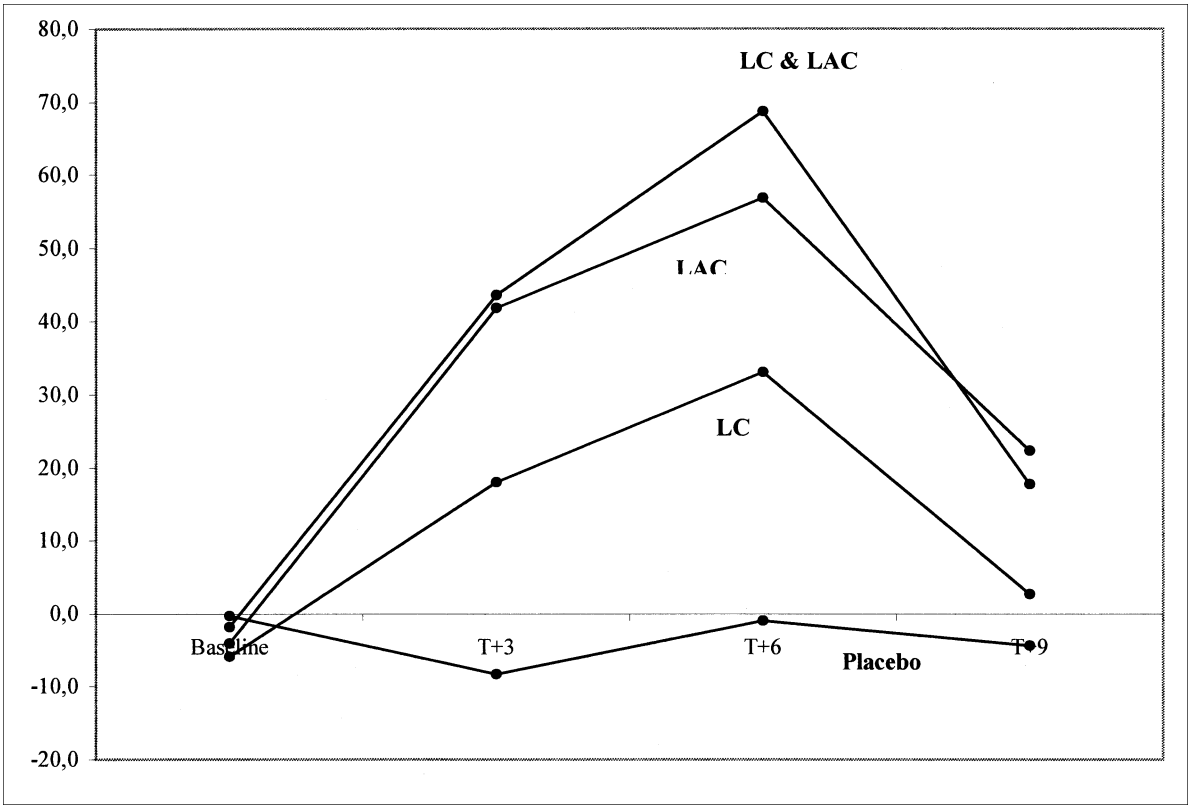
atypical sperm cells, semen volume, or VCL. On the contrary, the percentage variation of VSL between T0 and T+3 was statistically significantly higher in the subgroup C (LC + LAC) than in the placebo group (44.86 ± 76.24 vs. −4.59 ± 37.05; $F=3.077$; $P=.035$). (Note: Measures are expressed in percentage variations from T0.) In other words, patients treated with the combination of the two molecules improved significantly during the first 3-month period of the administration.

A statistically significant improvement in total sperm motility was found in patients to whom LAC was administered, either alone or combined with LC (from −3.3 ± 17.4 at T0 to 37.7 ± 27.8 at T+6; $F=11.19$; $P=.001$) (Fig. 2). The

FIGURE 1

Forward sperm motility at each time in the four treatment groups: percentage variations with respect to T-1.



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analysis of forward sperm cell motility showed the same results (from −2.9 ± 26 at T0 to 63 ± 66.8 at T+6; $F=12.68$; $P=.001$) (Fig. 3). (Note: Measures are expressed in percentage variations from T-1.)

An improvement of forward motility was found when LC + LAC was compared with LC only or LAC only, although the variations of kinetic sperm parameters were not statistically significant (see Fig. 1). No statistically significant modifications were found in placebo group.

In all carnitine therapy groups, a statistically significant dependence of the total and forward motility variations on the baseline values was found. Patients with lower baseline values of motility had a statistically significant higher probability to be responders to the treatment.

After washout (T+9), sperm cells kinetic features (total and forward motility, VSL) were statistically significantly reduced in treatment groups when compared with month T+6.

In the group to whom LAC was administered (alone or combined), the sperm concentration showed a statistically significant variation during the treatment period (from 6.95 ± 22.06 at T0 to 42.88 ± 50.80 at T+6; $F=3.611$; $P=.015$). (Note: Measures are expressed in percentage variations from T-1.)

A statistically significant reduction of atypical sperm cells was also evident between T0 and T+6. In particular, the improvement was statistically significantly different in the LC.

No statistically significant different variations in semen volume and in VCL were detected in the studied patients.

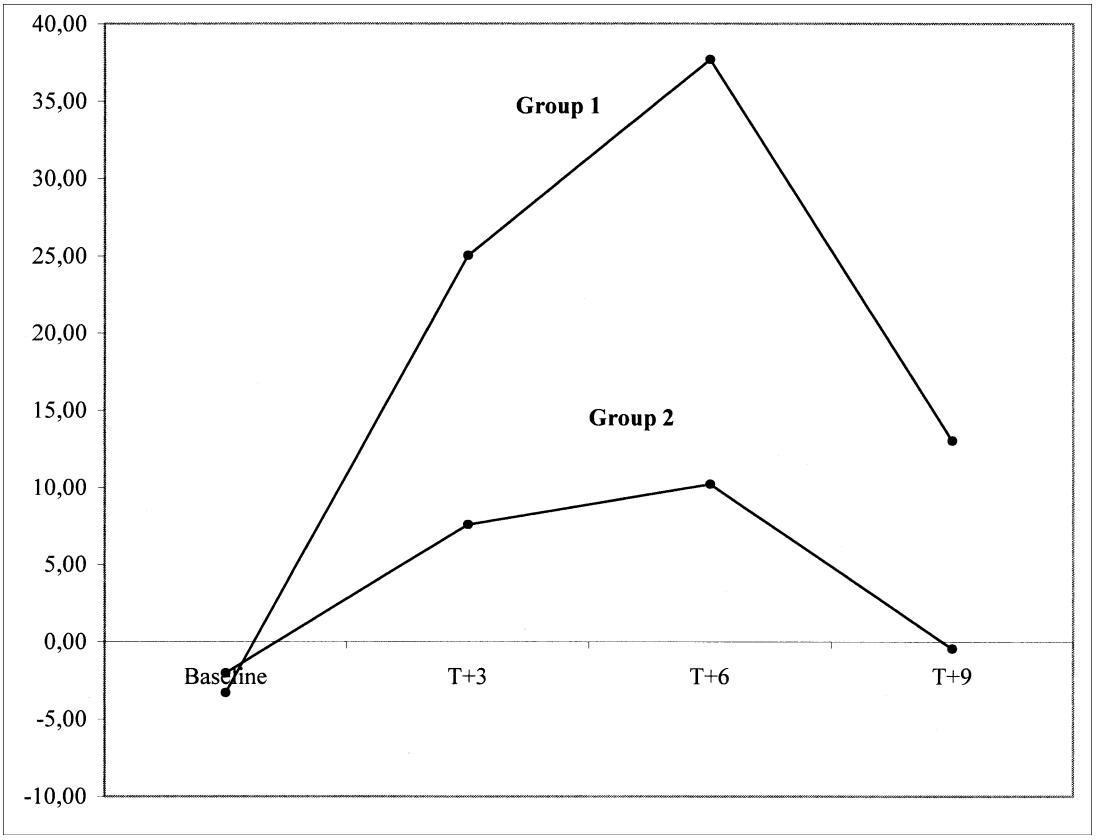
Total Oxyradicals Scavenging Capacity

Table 3 reports mean and standard deviation of TOSC values at each time and the percentage variations with respect to baseline. The TOSC assay of the seminal fluid toward different ROS showed a statistically significant improvement for both hydroxyl and peroxyl radicals in the treatment groups, but no statistically significant modifications were found in placebo group.

The increase in TOSC values, between T0 and T+6, was positively correlated with the improvement of kinetic features: with total motility, forward motility for both the radicals, and with VCL or VSL, respectively, for hydroxy

FIGURE 2

Total sperm motility at each time: percentage variations respect to T-1 (Time: $P<.001$; LACTX: $P=.001$). Group 1: patients treated with L-acetyl-carnitine (LAC), alone or combined. Group 2: patients treated with L-carnitine (LC) or placebo.



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radicals and peroxyl radicals (Table 4). Moreover, forward motility variation was found to depend on the baseline values of TOSC (hydroxyl radicals).

Spontaneous Pregnancies

Twelve spontaneous pregnancies were achieved during the observation period. Opening the randomization list revealed that nine of the patients who had impregnated their female partner had undergone carnitine therapy. Five of these pregnancies resulted from patients who had received LC + LAC therapy (three of which after 3 months, and two after 5 months of treatment). Two pregnancies had resulted from patients who had been given LC therapy (after 3 months of treatment). Two further pregnancies resulted from patients who had received LAC therapy (one after 2 months, one after 5 months of therapy). Three out of the 12 pregnancies occurred in partners of patients undergoing placebo treatment (after 1 month of therapy, after 3 months of therapy, and after 2 months of washout).

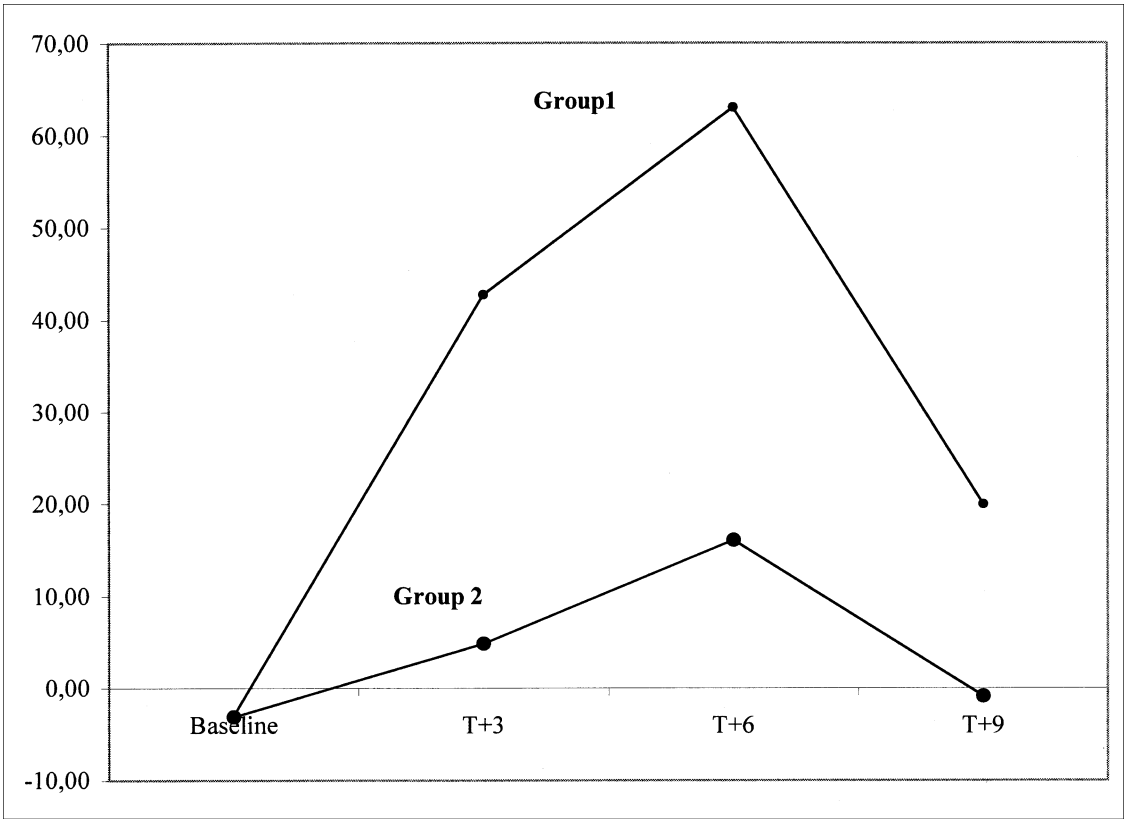
DISCUSSION

Several controlled and uncontrolled studies support a potential positive effect of therapy with LC and its acyl derivatives in selected forms of oligoasthenoteratozoospermia (21–25). In particular, a very recent controlled study reports the efficacy of LC and LAC combined treatment in improving sperm motility, especially in patients with lower baseline levels (25). The main rationale is based on the central role of carnitine in energetic metabolism and its accumulation in epididymal fluid and spermatozoa, both as free and acetylated LC (4). Although some evidence suggests a secondary role of carnitine as antioxidant (26), its effective role and mechanism of action still remain an interesting open question.

The detrimental effects of ROS and other pro-oxidant molecules on sperm function has been largely described (8–15); on the other hand, several data support a reduced total oxyradical scavenging capacity of seminal fluid in infertile men with abnormal semen parameters (18, 28, 29). Interestingly, the therapeutic use of antioxidant molecules

FIGURE 3

Forward sperm motility at each time: percentage variation respect to T-1 (Time: $P<.001$; LACTX: $P=.001$). Group 1: patients treated with L-acetyl-carnitine (LAC), alone or combined. Group 2: patients treated with L-carnitine (LC) or placebo.



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such as glutathione and coenzyme Q10 was shown to improve semen quality in infertile males (19, 20).

Our double-blind, randomized, placebo-controlled trial addressed the following points: [1] the effectiveness of various treatments in improving semen parameters and the comparison between the use of LC or LAC alone or LC-LAC combined treatment; [2] the different sensitivity of patients in relation to baseline semen parameters; and [3] variations in total oxyradical scavenging capacity and its correlation with semen parameters after treatment.

As far as the first point is concerned, the main goal of the study was the improvement of sperm cell motility, both considering the total motility and forward motility (including VSL determined by CASA analysis) in patients to whom LAC was administered both alone or in combination with LC. It is interesting to note that combined LC + LAC resulted in improved forward motility and VSL of spermatozoa, when compared with LC or LAC therapy alone. Although such improvement was statistically significant only for VSL at time T+3, we think it should be stressed from a biologic point of view, as the former parameters are important in male fertility (27).

The washout period with two semen analyses before the start of therapy minimized the effect of spontaneous variations in seminal characteristics and allowed the evaluation of therapeutic effects (27). The statistically significant reduction of sperm motility after 3 months of washout (from T+6 to T+9) supported the relationship of the benefit between semen parameter improvement and carnitine treatment. It seems reasonable to relate it to the lack of the positive effect of carnitine both on sperm cell energy metabolism and on the scavenging capacity of seminal fluid after therapy suspension.

Interestingly, the efficacy of therapy seems to differ depending on the baseline kinetic values, the lower ones being predictive of a better response to the therapy. The present data are in full accordance with reports of other investigators in two recent papers (24, 25).

The improvement of the scavenging capacity of seminal fluid on hydroxyl and peroxyl radicals in treated patients,

TABLE 3

Descriptive statistics of total oxyradical scavenging capacity (TOSC) variables at each time: absolute and percentage variations, mean ± standard deviation.

TOSC	Treatment	Month T0	Month T + 6	Absolute variations	Percentage variations
Hydroxyl					
	PLAC	31,276.67 ± 5467.22	28,931 ± 5351.48	−2345.53 ± 3516.51	−7.12 ± 10.43
	LC	26,301 ± 6127.76	30,636.20 ± 5646.47	4334.67 ± 3454.27	18.52 ± 15.49
	LAC	27,566 ± 6139.02	31,645.79 ± 4680.91	4382.64 ± 3883.61	20.67 ± 27.19
	LC + LAC	27,207 ± 6061.33	31,712.67 ± 5933.07	4505.07 ± 2730.59	18.38 ± 12.72
Peroxyl					
	PLAC	24,879.20 ± 4865.76	24,358.20 ± 5466.47	−521.00 ± 1548.79	−2.43 ± 6.79
	LC	26,035.60 ± 4098.60	31,003.00 ± 5841.52	4967.40 ± 3900.30	19.48 ± 15.86
	LAC	22,775.73 ± 5675.95	24,924.07 ± 6344.44	2474.00 ± 3487.15	12.32 ± 21.72
	LC + LAC	25,899.00 ± 5147.45	29,889.13 ± 5345.76	3990.13 ± 2533.32	16.53 ± 12.64

Note: LC = L-carnitine; LAC = L-actylcarnitine; PLAC = placebo.

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according to the TOSC assay, is one of the most striking features of the present study. The scavenging capacity has been found to be positively related to the improvement of the kinetic parameter of sperm cells, and a statistically significant dependence of the forward motility variations on the baseline TOSC values toward hydroxyl radicals was evident.

Moreover, lower values of TOSC (hydroxyl radicals) seem to be predictive of a better response to carnitine administration. Our strongly support [1] the role of carnitine as an antioxidant; [2] a pathogenic involvement of the reduced oxyradical scavenging capacity in idiopathic asthenozoospermia, according to a previous report (18).

Why carnitine can act as an antioxidant remains an unsolved problem at present. From a biochemical point of view, it is not a direct ROS scavenger. We can only hypothesize an indirect mechanism, probably related to the reduction in lipid peroxidation (30–32), and protective effects on cell membranes and protein oxidation as well as pyruvate and lactate oxidative damage (33).

A statistically significant reduction of atypical cell morphologic features was also detected in LC-treated patients. A possible metabolic positive modification induced by the carnitine therapy directly on the cell gametes as well as the improved oxyradical scavenging capacity of the biologic system could speculatively explain such data.

Although pregnancy was not an end point for this controlled study, due to many possible interferences, it is interesting to note that nine pregnancies occurred in carnitine-treated patients

TABLE 4

Descriptive statistics of total oxyradical scavenging capacity (TOSC) variable at each time: correlations between the increase of TOSC values of hydroxyl or peroxyl radicals and sperm variables.

TOSC	Sperm variable	Pearson correlation	Significance (two-tailed)
Hydroxyl			
	Total motility	.391	.002
	Forward motility	.455	<.001
	Curvilinear velocity (VCL)	3.57	.006
Peroxyl			
	Total motility	.410	.001
	Forward motility	.439	.001
	Straight progressive velocity (VSL)	.316	.015

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during therapy and that five of them were achieved after combined LC + LAC administration. Considering the pregnancies in partners of placebo-treated patients, two of them occurred after 1 month of therapy and 2 months of washout, respectively; neither case was temporally related to the treatment.

All together, our data seem to suggest that long-term carnitine therapy is effective in improving sperm function and fertilization capacity. Following the end points of the study, we can conclude that [1] LC and LAC administration is effective in increasing sperm motility in patients affected by idiopathic asthenozoospemia, especially in those with lower baseline values and lower baseline total oxyradical scavenging capacity; combined LC + LAC therapy improves sperm cell forward motility (although not statistically significant) and VSL (statistically significant improvement) when compared with LC or LAC therapy alone; [2] the administration of both LAC and LC improves the total scavenging capacity of the seminal fluid in the same population.

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