

# A placebo-controlled double-blind randomized trial of the use of combined L-carnitine and L-acetyl-carnitine treatment in men with asthenozoospermia

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**Objective:** To determine the efficacy of combined L-carnitine and L-acetyl-carnitine therapy in infertile males with oligo-astheno-teratozoospermia.

**Design:** Placebo-controlled double-blind randomized trial.

**Setting:** University tertiary referral center.

**Patient(s):** Sixty infertile patients (aged 20–40 years) with the following baseline sperm selection criteria: concentration, 10 to 40 × 10<sup>6</sup>/mL; forward motility, <15%; total motility, 10% to 40%; and atypical forms, <80%. Fifty-six patients completed the study.

**Intervention(s):** Patients were submitted to a combined treatment of L-carnitine (2 g/d) and L-acetyl-carnitine (1 g/d) or of placebo; the study design was 2 months' wash-out, 6 months of therapy or of placebo, and 2 months' follow-up.

**Main Outcome Measure(s):** Variation in the semen parameters that were used for patient selection.

**Result(s):** Even though increases were seen in all sperm parameters after combined carnitine treatment, the most significant improvement in sperm motility (both forward and total) was present in patients who had lower initial absolute values of motile sperm (<4 × 10<sup>6</sup> forward or <5 × 10<sup>6</sup> total motile spermatozoa per ejaculate).

**Conclusion(s):** Combined treatment with L-carnitine and L-acetyl-carnitine in a controlled study of efficacy was effective in increasing sperm motility, especially in groups with lower baseline levels. (Fertil Steril® 2004; 81:1578–84. ©2004 by American Society for Reproductive Medicine.)

**Key Words:** Male infertility, asthenozoospermia, semen, carnitine, sperm motility

Free L-carnitine (3-hydroxy-4-N-trimethylaminobutyric acid) has a pivotal role in cellular energy production. It was first isolated from bovine muscle in 1905, and its structure was established in 1927. Free L-carnitine is necessary in mitochondrial  $\beta$ -oxidation of long-chain fatty acids. Fatty acids must undergo activation (bind to coenzyme A [CoA] to form acyl-CoA) to enter the mitochondria. Long chain acyl-CoA molecules are incapable of crossing the internal mitochondrial membrane and hence use a specific enzymatic mechanism, which uses L-carnitine as a shuttle (1–3). After transport into the mitochondria, acyl is transferred to the mitochondrial CoA by acyl-carnitine; this initiates  $\beta$ -oxidation with the product

adenosine triphosphate and finally exits as L-acetyl-carnitine to start a new transport cycle.

Carnitine also protects the cell membrane and DNA against damage induced by free oxygen radicals. It prevents protein oxidation and pyruvate and lactate oxidative damage (4).

Seventy-five percent of the carnitine that is present in humans derives from diet, whereas 25% is synthesized from lysine and methionine, even though the enzyme that catalyzes the hydroxylation of the 4-butirrobetain in L-carnitine, 4-butirrobetain hydroxylase, is present in few tissues (5). The highest levels of L-carnitine in the human body are found in epididymal fluid, in which its concentration is around

2,000 times higher than in circulating blood (2 to 100 mM vs. 10 to 50  $\mu$ M).

The initiation of sperm motility occurs in parallel to an increase in carnitine concentration in the epididymal lumen and L-acetyl-carnitine in spermatozoa (2, 6, 7). Epididymal epithelium secretes L-carnitine into the lumen by specific active transport systems (8).

Taking into account their mechanisms and functions, L-carnitine and L-acetyl-carnitine have been proposed and used as a possible treatment in selected forms of oligoasthenoteratozoospermia (9–12).

We report here on a 6-month double-blind, randomized placebo-controlled trial of combined L-carnitine and L-acetyl-carnitine treatment in a selected group of infertile male patients who had various degrees of oligoasthenoteratozoospermia.

## MATERIALS AND METHODS

### Study Design

The study was authorized by the Italian Ministry of Health. The study was approved by the Institutional Review Board and Ethical Committee of the Faculty of Medicine at Rome University Hospital.

A group of 60 patients were submitted to the following: double-blind therapy of L-carnitine (10 mL vial containing 2 g/d orally of Carnitene; Sigma Tau, Pomezia, Rome, Italy) and L-acetyl-carnitine (tablet containing 500 mg twice per day orally every 12 hours; Zibren, Sigma Tau) or a seemingly identical placebo (each 10-mL placebo vial contains the following: malic acid, sodium benzoate, sodium saccharinate dihydrate, anhydrous sodium citrate, pineapple flavoring, demineralized water; each placebo tablet contains a core with 1-hydro lactose, magnesium stearate, polyvinylpyrrolidone, corn starch, and a coating with cellulose acetophthalate, dimethicone, ethyl phthalate; Sigma Tau).

The carnitine dose chosen was both the most commonly used dosage for treatment of other diseases (kidney, nervous system, heart, muscular disease, and so on) and similar to that used in past trials on male infertility by our group and other investigators (9–12).

The study design was 2 months' wash out, 6 months' therapy (30 patients) or placebo (30 patients), and a further 2 months' follow-up (controls at months T–2, T0, T+3, T+6, T+8).

Monthly evaluation of two semen samples before the beginning of treatment (T–2, T0) was carried out to test semen parameter stability in each patient as recommended by the World Health Organization (13). At time T+3, only one semen sample was collected per patient; at T+6, two consecutive semen samples after 5 days of abstinence were collected; and at T+8, only one semen sample was collected per patient.

The double semen analysis was performed only at T+6, which, being the end of the treatment period, was considered as the most relevant period.

At various time points the following analyses were carried out:

Microscopic semen analysis (T–2, T0, T+3, T+6, T+8) to evaluate modifications in semen and sperm parameters (13), seminal carnitine concentration (T0 and T+6) to evaluate possible concentration variations during therapy (14), and sperm lipid peroxidation potential (LPOp) evaluated by thiobarbituric acid assay (T0 and T+6) to test possible sperm membrane variations (15).

All analyses were performed on both therapy and placebo patients.

Patient compliance and possible side effects were also noted, and blood analyses were carried out at T0 and T+6 to evaluate the treatment's safety.

On the basis of previous results from our group and other authors (9–12), improvement in sperm motility (both total and forward) was considered to be the main measurement of efficacy.

### Semen Analysis

All microscopic semen analyses (six for each patient) were carried out by the same biologist (L.G.) by World Health Organization standard procedures (13) and our own standards. The microscopic equipment Leica DMRA (Wetzlar, Germany) was used for all semen analyses. Our laboratory is accredited by the Italian Institute of Health as the guide laboratory for national external quality control (EQC) in seminology (16) and is assessed by an international EQC (UKNEQAS).

Samples were collected by masturbation after 3–5 days of sexual abstinence. Semen variables taken into consideration were the following: ejaculate volume (in milliliters), sperm concentration ( $n \times 10^6$ /mL), total sperm count ( $n \times$  volume of ejaculate), forward progressive sperm motility (percentage at 1 hour after ejaculation), total motility (percentage of linear progressive and nonlinear and nonprogressive) and sperm morphology (percentage of atypical forms). Sperm morphology was evaluated by the May Grünwald Giemsa staining procedure.

Absolute values for total and forward-motile spermatozoa per ejaculate were also calculated by multiplying the percentage of total or forward sperm motility by sperm concentration per milliliter and by the volume (in milliliters) of the single ejaculate. This enabled a more reliable evaluation of the effect of treatment on sperm motility in cases of severe oligozoospermia or asthenozoospermia, in which the varia-

tion in sperm motility percentage is possibly not sensitive enough as an indicator.

## Study Group and Eligibility

The study group of 60 patients was selected by a single andrology team, on the basis of sample size calculation. Written, informed consent was obtained from all participants. General inclusion criteria were as follows: age, 20–40 years; infertility, >2 years; and regular sexual intercourse with a fertile female. The female partner was defined as being fertile if she had undergone a negative infertility workup (biphasic basal body temperature, P evaluation in luteal phase, ultrasound ovary and uterus evaluation, and hysterosalpingogram to study tubal patency).

Clinical inclusion criteria for the study subjects were the absence of the following:

General and endocrinological diseases (clinical examination and routine and hormonal laboratory tests), present or previous cryptorchidism, genital infections or genital tract obstructions (sperm culture, urethral swab mycoplasma and chlamydia test, and biochemical study of seminal plasma), varicocele and testicular hypotrophy (ultrasonography and Doppler color flow), and antisperm antibodies (tested both in sera and bound to the sperm surface) (17).

Patients were requested to follow a standard diet to avoid effects attributable to variable L-carnitine intake in food. None of the patients suffered from L-carnitine metabolism deficiency.

Semen inclusion criteria were as follows: normal rheological characteristics (appearance, consistency, and liquefaction), volume and pH in the normal range, sperm concentration of  $10$  to  $40 \times 10^6/\text{mL}$ ; forward motility, <15%; total motility, 10%–40%; atypical forms, <80%; semen leukocytes, < $1 \times 10^6/\text{mL}$ . These upper and lower sperm parameter limits allowed the inclusion of cases of mild oligoasthenoteratozoospermia and severe asthenozoospermia and teratozoospermia. These were chosen on the basis of previous experience (11). The sperm concentration limits allowed the exclusion of cases of severe quantitative spermatogenesis defect (< $10 \times 10^6/\text{mL}$ ) that would have precluded observation of positive or negative effects of the treatment.

For inclusion in the trial, patients had to meet the above semen inclusion criteria at both wash-out times (T–2 and T0). This excludes any patient with a transient decrease in semen quality during the wash-out period who then has a sudden (and treatment-independent) improvement at subsequent observation points.

## Statistical Analysis

Means, SD, and SE were calculated on all clinical and seminal variables at each time control.

Independent-samples *t* test was used to evaluate the significance of differences between the variables that were observed at the two wash-out semen analyses (T–2 and T0).

Student's *t*-test was used to evaluate the homogeneity between “placebo” and “therapy” patient groups at the baseline (T0).

In every stage of statistical analysis, results reported for T+6 are the mean of values obtained from the two consecutive semen samples collected after the 6th month of treatment (therapy or placebo).

Variance analysis for repeated measures was used to evaluate any significant variation in quantitative variables over time between the therapy and placebo groups. The *between* factor was the difference between placebo and therapy treatment, and the *within* factor was time, with a first analysis of five levels (T–2, T0, T+3, T+6, and T+8) and a second of four levels (T0, T+3, T+6, and T+8).

A *t* test for independent samples (on differences between T+6 and T0) was used to evaluate whether the overall semen parameter variation was significantly different between the two groups.

Linear regression models (T+6 – T0/T0) were used to study the significance of variation between placebo and therapy patient groups for sperm concentration, motility (forward and total), morphology, and seminal carnitine concentration.

On the basis of previous experience (11), both raw data (e.g., forward and total motility percentages) and absolute values in terms of millions of motile spermatozoa per ejaculate (obtained by multiplying the total sperm per ejaculate by the percentage of total and forward sperm motility) were subjected to statistical analysis. This was performed by the Mann-Whitney *U* test, analyzing and comparing therapy and placebo patients both on the whole group and after division into subgroups on the basis of the number of total and forward-motile spermatozoa at time T0: that is, < $20 \times 10^6$ , < $10 \times 10^6$ , and < $5 \times 10^6$  total motile sperm per ejaculate and < $12 \times 10^6$ , < $8 \times 10^6$ , and < $4 \times 10^6$  forward-motile sperm per ejaculate.

These subgroups were chosen arbitrarily, with the only criterion being a homogenous distribution of cases (in both therapy and placebo group) in each one, with the aim of verifying previous observations (11) on the improvement in sperm motility in groups with lower initial values. This allowed detection of a possible optimal threshold of variable sperm motility (total and forward) in which the maximum therapeutic effect was achieved.

## RESULTS

Four patients dropped out from the study. When the codes were broken at the end of the study, it was seen that all 30 patients included in the therapy group, and 26 of the 30

**TABLE 1**

Descriptive analysis of the semen parameters of all selected patients, divided into therapy group (30 patients) and placebo group (26 patients).

Parameter	T-2	T0	T+3	T+6	T+8
<b>Therapy group</b>					
Vol (mL)	3.25 ± 1.24	3.13 ± 1.42	3.12 ± 1.17	3.17 ± 1.50	3.15 ± 1.53
Conc (n × 10 <sup>6</sup> /mL)	19.60 ± 6.56	18.07 ± 5.68	21.15 ± 9.69	22.09 ± 9.05	18.23 ± 9.31
Mot tot (%)	24.50 ± 5.31	23.17 ± 6.50	28.18 ± 11.40	31.11 ± 13.46	27.56 ± 9.22
Mot frw (%)	15.67 ± 4.50	14.83 ± 5.17	20.91 ± 11.41	25.00 ± 13.06	19.44 ± 9.06
Atyp (%)	76.17 ± 4.62	76.87 ± 4.10	75.12 ± 6.70	75.59 ± 7.79	77.18 ± 5.86
<b>Placebo group</b>					
Vol (mL)	3.00 ± 1.19	2.75 ± 1.16	3.16 ± 1.67	3.14 ± 1.47	3.03 ± 1.65
Conc (n × 10 <sup>6</sup> /mL)	15.58 ± 7.40	16.12 ± 7.79	19.82 ± 13.92	22.17 ± 16.95	19.67 ± 11.09
Mot tot (%)	24.23 ± 5.23	23.08 ± 6.49	25.77 ± 9.45	29.55 ± 9.50	25.00 ± 9.26
Mot frw (%)	16.35 ± 4.37	15.42 ± 5.35	19.62 ± 9.79	24.41 ± 9.31	18.41 ± 9.43
Atyp (%)	76.00 ± 4.94	75.42 ± 5.22	73.86 ± 8.85	71.11 ± 9.10	74.94 ± 8.78

Note: Data are mean ± SD. All *P* values are >.05. Vol = semen volume; Conc = sperm concentration; Mot tot = percentage of total sperm motility; Mot frw = percentage of forward sperm motility; Atyp = percentage of atypical forms.

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patients included in the placebo group completed the study. **Table 1** reports mean and SD of sperm variables at each time.

The two semen analyses conducted before treatment (T-2 and T0) showed no statistically significant differences in *t* test for independent samples conducted on semen volume, sperm concentration, motility (total and forward), and morphology. Values measured at the beginning of the treatment (T0) were therefore acceptable as the baseline for further comparisons.

Results at T+3 and the mean of the two analyses at T+6 compared by analysis of variance for repeated measures did not show statistically significant differences for either single patients or the placebo and therapy groups. Mean values at T+6 were then used in the further comparison with values at T0.

Subjects undergoing carnitine therapy showed no improvement in semen volume and sperm concentration and

percentage of normal morphology but did have an improvement in total and forward sperm motility as compared with placebo patients (from 23.17 ± 6.50 to 31.11 ± 13.46 and from 14.83 ± 5.17 to 25.00 ± 13.06). However, the differences between the therapy and placebo groups for all the semen variables analyzed were not statistically significant for either analysis of variance for repeated measures or *t* test for independent samples (on differences between T+6 and T0). Neither did the linear regression analysis model show a statistical significance in the variation observed.

Further analyses were carried out on absolute values in millions of total motile spermatozoa present in the ejaculate. A significantly higher increase was observed in the therapy than in the placebo group when comparing T0 and T+6 values for the whole group (**Tables 2 and 3**) by Mann-Whitney test (*P* = .042).

In addition, the significantly higher increase in total motile sperm present in the ejaculate was seen when comparing

**TABLE 2**

Analysis of total motile sperm per ejaculate (n × 10<sup>6</sup>) and forward motile sperm per ejaculate (n × 10<sup>6</sup>) in therapy group (30 patients) and placebo group (26 patients).

Parameter	T-2	T0	T+3	T+6	T+8
<b>Therapy group</b>					
Total motile sperm/ejaculate	11.185 ± 6.414	11.876 ± 8.450 <sup>a</sup>	21.887 ± 16.971	30.654 ± 21.792 <sup>a</sup>	17.251 ± 8.658
Forward motile sperm/ejaculate	7.411 ± 4.151	7.456 ± 5.949 <sup>b</sup>	17.755 ± 14.277	25.149 ± 24.340 <sup>b</sup>	13.770 ± 6.487
<b>Placebo group</b>					
Total motile sperm/ejaculate	15.503 ± 7.748	13.528 ± 9.943 <sup>a</sup>	17.944 ± 12.869	24.153 ± 13.558 <sup>a</sup>	16.544 ± 15.798
Forward motile sperm/ejaculate	10.084 ± 5.838	8.647 ± 6.980 <sup>b</sup>	13.801 ± 11.203	19.870 ± 11.313 <sup>b</sup>	12.488 ± 13.873

Note: Data are mean ± SD.

<sup>a,b</sup> Mann-Whitney *U* test significance (*P* values between T0 and T+6): <sup>a</sup>*P* = .042; <sup>b</sup>*P* = .044.

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TABLE 3

Analysis of motile spermatozoa variation before and after treatment (T0 and T+6) in patients included in the individual subgroups, identified by total motile sperm per ejaculate ( $n \times 10^6$ ) and forward motile sperm per ejaculate ( $n \times 10^6$ ) at T0.

Sperm count groups	Total motile sperm per ejaculate		Sperm count groups	Forward motile sperm per ejaculate	
	Time			Time	
	T0	T+6		T0	T+6
Therapy group					
<20 × 10 <sup>6</sup>	18.5 ± 3.4	26.3 ± 6.2	<12 × 10 <sup>6</sup>	10.7 ± 0.8	23.1 ± 5.5
<10 × 10 <sup>6</sup>	8.4 ± 2.6	12.5 ± 3.9	<8 × 10 <sup>6</sup>	6.3 ± 2.1	12.4 ± 4.8
<5 × 10 <sup>6</sup>	3.4 ± 1.9	6.9 ± 3.2 <sup>a,b</sup>	<4 × 10 <sup>6</sup>	2.7 ± 1.6	7.5 ± 2.3 <sup>c,d</sup>
Placebo group					
<20 × 10 <sup>6</sup>	19.2 ± 2.7	26.1 ± 3.1	<12 × 10 <sup>6</sup>	9.3 ± 1.3	21.2 ± 6.5
<10 × 10 <sup>6</sup>	7.6 ± 2.9	10.6 ± 2.8	<8 × 10 <sup>6</sup>	7.1 ± 1.2	10.3 ± 3.8
<5 × 10 <sup>6</sup>	2.7 ± 2.8	3.4 ± 2.2 <sup>a,b</sup>	<4 × 10 <sup>6</sup>	2.5 ± 0.8	4.5 ± 2.2 <sup>c,d</sup>

<sup>a,b,c,d</sup> Mann-Whitney U test and proportion of variation significance between T0 and T+6 in the patient subgroups, with <5 × 10<sup>6</sup> of total motile sperm per ejaculate at T0 (<sup>a</sup>*P*=.038; <sup>b</sup>*P*=.018) and with <4 × 10<sup>6</sup> of forward motile sperm per ejaculate at T0 (<sup>c</sup>*P*=.043; <sup>d</sup>*P*=.028).

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the therapy and placebo subgroups with T0 values of <5 × 10<sup>6</sup> total motile sperm per ejaculate. This was evaluated both by the Mann-Whitney test (*P*=.038) and by the analysis of the proportion of variation of total motile spermatozoa, which was significantly higher in treated than in placebo patients (*P*=.018).

The absolute values of forward-motile spermatozoa, expressed as millions of forward-motile spermatozoa present in the ejaculate, also showed a significantly higher increase in the therapy group comparing T0 and T+6 values in the whole groups (Tables 2 and 3) by Mann-Whitney *U* test (*P*=.044). Furthermore, at T+6, a significantly higher increase of forward sperm motility was observed in patients with a value at T0 of <4 × 10<sup>6</sup> forward-motile sperm per ejaculate.

This was evaluated by both the Mann-Whitney test (*P*=.043) and the analysis of the proportion of variation of forward-motile spermatozoa, which was significantly higher in treated than in placebo patients (*P*=.028). In this same group of patients (<4 × 10<sup>6</sup> forward-motile sperm per ejaculate), the proportion of variation of total motile sperm per ejaculate was also significantly higher during the therapy period (*P*=.015), as confirmed by the Mann Whitney test (*P*=.029).

Four spontaneous pregnancies were achieved during the observation period. After the codes were broken, it was found that all patients who initiated a pregnancy in their female partner had undergone carnitine therapy. Evaluation of female partner menstrual history showed that all fecundations were achieved during the period of carnitine therapy (two at the 4th month of therapy, one at the 5th month, and one at the 6th month).

Interestingly, all these patients were in the subgroups with higher T0 values of forward-motile sperm per ejaculate: two pregnancies were induced by patients with <8 × 10<sup>6</sup> forward-motile sperm per ejaculate and two by patients with <12 × 10<sup>6</sup> forward-motile sperm per ejaculate. None of these patients showed a statistically significant variation of forward sperm motility between T0 and T+6.

No statistically significant variation was observed in seminal carnitine concentration and LPOp between T0 and T+6.

## DISCUSSION

Free L-carnitine is much more concentrated at the epididymal level than in blood. In the epididymis, free L-carnitine is transported from blood plasma into the epididymal fluid and spermatozoa and accumulates as both free and acetylated L-carnitine. The use of L-carnitine and its acyl derivatives in therapy has been proposed in recent years for treatment of oligoasthenoteratozoospermia of unknown origin. A number of controlled and uncontrolled studies seem to indicate their possible application, with the rationale for their actions on sperm epididymal maturation and energetic metabolism being an intracellular mechanism and that for their actions on the testicular-epididymal microenvironment being an indirect antioxidant action. Even though many positive results have been published (9–12), many questions still remain open.

In our current double-blind, randomized, placebo-controlled trial we have examined some of the questions that were raised in our previous article (11): [1] whether combined L-carnitine and acetyl-L-carnitine therapy is effective in cases of oligoasthenoteratozoospermia; [2] whether the improvement in sperm motility is sustained over a longer

therapy period; and finally, [3] whether there is a difference in response among subgroups of patients.

The wash-out period with two semen analyses before the start of therapy minimized the possible effect of spontaneous variations in seminal characteristics while allowing evaluation of the therapeutic effect (13), and the two consecutive semen evaluations at T+6 reduced the possibility of variations independent of the therapy.

We used a 6-month therapy or placebo period (instead of the 2 + 2 month therapy/placebo cross-over design with wash-out periods before and between treatments of the previous trial) to focus on the possible effect of acetyl and L-carnitine on phases of spermatogenesis, as well as to understand the reasons for a slight increase in sperm concentration that was observed elsewhere after 2 months of L-carnitine therapy (11).

No significant variation in sperm concentration was observed after 3 or 6 months of therapy, thus excluding a direct positive effect on the spermatogenic process. The lack of any change in sperm morphology (considered the most sensitive index of spermatogenesis) confirms the hypothesis of a post-testicular effect.

Individual subgroups selected on the basis of the number of motile spermatozoa indicate that the therapy's action could differ according to the basis of semen characteristics at the start of treatment.

The positive effect obtained on all sperm variables that were also in the placebo group confirms that an important psychological element exists, even in infertility. This may be related to the sensation of being treated and the continuous availability of medical staff and counseling. In fact, this result could be only in part attributable to the statistical phenomenon of regression to the mean.

The absence of statistically significant differences between mean values at T+3 and at T+6 demonstrates the stability of the improvement achieved. This longer treatment in comparison with our previous trial (11) does not seem to further improve these results.

However, differences related to the length of the therapy period were observed in the pregnancies obtained. In fact, although pregnancy was not the principal end point for this controlled study because of the many possible interfering factors, four patients taking carnitine induced a pregnancy in their partner. None of these patients showed a significant improvement in sperm parameters. All these pregnancies were observed after the 4th month of combined carnitine treatment. This result suggests that long-term combined carnitine treatment may improve sperm function and fertilization capacity in ways that are not evident from microscopic analysis of sperm parameters.

This also seems to be confirmed by the observation that seminal carnitine concentration and lipid peroxidation po-

tential did not show a significant increase during therapy. Because carnitine acts at an intracellular level, this suggests a possible molecular and metabolic modification induced by the therapy directly on the cell gametes.

Apart from the expected action on energy production, we can hypothesize a specific action on mitochondrial membrane structure and potential intracellular free radical defense mechanisms (DNA structure and chromatin integrity). All these possibilities must be investigated in future studies.

The parameter that was expected to undergo the most improvement in this study was sperm motility. It was therefore very interesting to observe the differences between the placebo and therapy groups, which became statistically significant when total and forward-motile spermatozoa present in the ejaculate were analyzed and also when subgroups with lower initial values for total and forward motility were considered. These last patients could be the highest responders in terms of motility when high doses of carnitine are added to their diet, which would correct the possible biochemical deficiency in their mitochondrial metabolism.

Finally, the pregnancies achieved, together with the improvement in numbers of motile sperm (both total and forward) that was observed in the subgroups with severe asthenozoospermia, could indicate the efficacy of combined L-carnitine and L-acetyl-carnitine treatment on single sperm subpopulations present in the semen. The therapy may amplify and boost these sperm subpopulations, acting directly on their maturation and energy metabolism or through an improvement in the testicular-epididymal microenvironment.

In conclusion, the combined L-carnitine and L-acetyl-carnitine treatment in a controlled study of efficacy was effective in increasing sperm motility, especially in groups with lower baseline levels. Further studies should concentrate on the sperm-fertilizing ability of patients with very low numbers of motile sperm who are undergoing assisted reproduction. Also needed are biological studies of the effect of carnitine on the metabolism of the male gamete, using molecular and cellular studies on single intracellular functions or organelles.

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