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Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial

Andrea Lenzi, M.D., Francesco Lombardo, Ph.D., Paolo Sgrò, M.D., Pietro Salacone, M.D., Luisa Caponecchia, B.Sc., Franco Dondero, M.D., and Loredana Gandini, B.Sc.

Outpatient Department and Laboratory of Seminology and Reproductive Immunology at the Training Center in Andrology of the European Academy of Andrology, and Department of Medical Physiopathology, University of Rome "La Sapienza," Policlinico Umberto, Rome, Italy

Objective: To determine the efficacy of L-carnitine therapy in selected cases of male factor infertility.

Design: Placebo-controlled, double-blind, crossover trial.

Setting: University tertiary referral center.

Patient(s): One hundred infertile patients (ages 20–40 years) with the following baseline sperm selection criteria: concentration, $10–20 \times 10^6$/mL; total motility, 10%–30%; forward motility, <15%; atypical forms, <70%; velocity, 10–30 μ/s; linearity, <4. Eighty-six patients completed the study.

Intervention(s): Patients underwent L-carnitine therapy 2 g/day or placebo; the study design was 2 months of washout, 2 months of therapy/placebo, 2 months of washout, and 2 months placebo/therapy.

Main Outcome Measure(s): Variation in sperm parameters used in the patients selection criteria, in particular, sperm motility.

Result(s): Excluding outliers, a statistically significant improvement in semen quality, greater than after the placebo cycle, was seen after the L-carnitine therapy for sperm concentration and total and forward sperm motility. The increase in forward sperm motility was more significant in those patients with lower initial values, i.e., $<5 \times 10^6$ or $<2 \times 10^6$ of forward motile sperm/ejaculate or sperm/mL.

Conclusion(s): Based on a controlled study of efficacy, L-carnitine therapy was effective in increasing semen quality, especially in groups with lower baseline levels. However, these results need to be confirmed by larger clinical trials and in vitro studies. (Fertil Steril® 2003;79:292–300. ©2003 by American Society for Reproductive Medicine.)

Key Words: Fertility, male infertility, oligozoospermia, semen, spermatozoa, therapy, L-carnitine, sperm motility, mitochondria

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Reprint requests: Professor Andrea Lenzi, Laboratory of Seminology and Reproductive Immunology, Andrology Unit, Department of Medical Physiopathology, University of Rome "La Sapienza," Policlinico Umberto, I-00161 Rome, Italy (FAX: 39-06-49970717; E-mail: andrea.lenzi@uniroma1.it).

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Birth rates in western countries are dropping rapidly. The causes of this decline are very complex and include, above all, sociological changes (e.g., urbanization, pollution, older parents). A real increase in cases of infertility was also postulated as a consequence of a possible, although not yet completely demonstrated, decline in semen quality (1–4). In fact, around 20% of couples have to wait more than 12 months, the time proposed by the World Health Organization (WHO) as the maximum normal limit, before achieving pregnancy. Male factor infertility represents around half of the general problem of infertility and is today a great health and social problem in terms of both prevention and therapy (5).

A man may have a longstanding infertility problem that he becomes aware of only when he remains childless despite unprotected intercourse. When a couple remains without a pregnancy long enough to define the couple as infertile, and semen analysis shows a sperm deficiency, the need for diagnosis and therapy for the male becomes evident. Excluding some cases with a specific and recognizable etiology (genetic, hormonal, infective, etc.), one of the most difficult problems we face is to translate our knowledge of the physiology of spermato-

genesis and sperm maturation into a rationale for treatment of male infertility (6–10); in many cases, even extensive clinical and laboratory screening might not result in a diagnosis (5).

A number of drugs have been proposed as being possible causes of male factor infertility associated with oligoasthenoteratozoospermia (OAT) of unknown origin. However, very few controlled studies have been carried out because of the difficulties in selecting patients and in obtaining a study group large enough to give statistically significant results (11–22).

In consequence, both general practitioners and specialists (andrologists, endocrinologists, urologists, gynecologists) around the world frequently employ, for the purpose of improving sperm quality, drugs of dubious efficacy based on anecdotal indications and without consideration for good medical practice.

We have studied, using a controlled trial, the effect of antioxidant therapies on sperm maturation and on the testicular-epididymal microenvironment (16). We observed positive results deriving from a possible effect on epididymal spermatozoa (23–25). The epididymis therefore seems to be a possible target of therapies acting on spermatozoa in cases of idiopathic OAT. The physiological role of the epididymis is to be active in spermatozoa metabolism through the many compounds secreted or produced by the epithelium; among these, carnitines are accumulated as both free and acetylated L-carnitine and are used by spermatozoa for mitochondrial beta-oxidation of long-chain fatty acids, this being the principal shuttle and transfer system of the acyl to the mitochondrial CoA (26, 27). Carnitine also acts on the cell DNA and membranes, protecting them against damage induced by free oxygen radicals (28).

Furthermore, at the beginning of the 1990s, a pilot multicenter uncontrolled study testing the efficacy of L-carnitine in selected cases of OAT, in which our group participated, showed some positive results on sperm motility (29), and a recent controlled study on the use of L-carnitine and L-acetyl-carnitine in patients with male genital tract inflammation showed that carnitines are an effective treatment in patients with abacterial prostatovesiculoepididymitis and elevated free oxygen radical production, even when seminal white blood cell concentration is normal (30).

In this paper, we report on a randomized placebo-controlled, double-blind, crossover trial of L-carnitine in a strictly selected group of infertile male patients suffering from OAT.

MATERIAL AND METHODS

Study Design

The study was approved by and was performed under the control of the Italian Ministry of Health and the Institutional Review Board (Ethical Committee) of the Faculty of Medicine at Rome University Hospital.

Patients were submitted to a therapy of L-carnitine (2 g/day orally of carnitene sigma tau; Pomezia, Rome, Italy) or an equal volume of seemingly identical placebo. The L-carnitine dose was selected to be similar to that used in past trials on this subject (29) and to be the most commonly used dosage in other diseases in which L-carnitine has been shown to be active (kidney, heart, muscular disease, etc.). The study design was 2 months of washout, 2 months of therapy/placebo, 2 more months of washout, 2 more months of placebo/therapy, and 2 months of follow-up (controls at months $T - 2$, $T - 1$, T_0, $T + 2$, $T + 4$, $T + 6$, and $T + 8$). Monthly evaluation of three semen samples before the beginning of treatment ($T - 2$, $T - 1$, T_0) was carried out to test semen stability in each patient as suggested by WHO (31). Although it would have been interesting, it was not possible to carry out multiple semen analyses for each control after the single periods of treatment and after the follow-up ($T + 2$, $T + 4$, $T + 6$, $T + 8$) for reasons of both patient compliance and submission to the advice of our Ethical Committee.

For each control, the following analyses were carried out: [1] complete microscopic and computer semen analysis (31), to evaluate semen and sperm parameter modifications; [2] seminal carnitine concentration (32), to evaluate possible variation in its concentration during therapy; [3] seminal α-glycosidase concentration (31), to evaluate the possible variation of an epididymal function index; [4] sperm lipid peroxidation potential (LPOp evaluated by thiobarbituric acid assay), to test possible variation in sperm membrane (33). Patient compliance and possible side effects were also noted.

Improvement in sperm variables was the primary efficacy parameter of this study, and, among such parameters, improvement in sperm motility (both total and forward) was considered as the main measurement of success. This was on the basis of both the expected effect of L-carnitine on sperm metabolism and the results of our previous experiences (29), which were confirmed by recent findings (30). However, although pregnancy was not considered to be a principal end point, as it is difficult to avoid the many confounding variables acting on naturally induced fertilization and subsequent pregnancy, we recorded pregnancies induced during the entire observation period and their assumed time of spontaneous fertilization (on the basis of the last ovulatory period of the female partner before the first β-hCG positive result). This was done both to give a secondary efficacy parameter and also to allow inclusion of results in a future meta-analysis in this field using pregnancy rate as an efficacy parameter.

Semen Analysis

All microscopic semen analyses (seven for each patient) were carried out by the same biologists using WHO (31)

standard procedures and our own standards. Our laboratory is accredited by the Italian Institute of Health as the guide lab for national External Quality Control (EQC) in seminology (34) and is assessed by an international EQC [United Kingdom National External Quality Assessment Scheme (UKNEQAS)]. Computer-assisted sperm motility analysis (CASA) was carried out taking account of WHO (31) standards and our own standards (35) using the HTM-Ivos system (Hamilton Thorne Research, Beverly, MA).

Samples were collected by masturbation after a 3- to 5-day period of sexual abstinence. Semen variables taken into consideration were volume (mL) and pH of ejaculate, sperm concentration ($n \times 10^6$/mL), total sperm number ($n \times$ volume of ejaculate), total and forward sperm motility (percent 1 hour after ejaculation), sperm morphology (percent of atypical forms), sperm velocity (μm/s), and linearity (index).

Total motile spermatozoa/mL and spermatozoa/ejaculate and total forward motile spermatozoa/mL and spermatozoa/ejaculate were also calculated by multiplying the percent of total or forward sperm motility, respectively, by [1] sperm concentration/mL and [2] total sperm number per ejaculate.

Study Group and Eligibility

The study group was selected by a single andrological team from more than 1,000 subjects visiting our outpatient department (from the end of 1998 to the beginning of 2000) for their first consultation relating to male factor infertility. We selected a group of 100 patients on the basis of a power calculation of effect size made by Ethical Committe statisticians. Written informed consent was obtained from all participants. The general inclusion criteria for enrollment were age between 20 and 40 years, infertility lasting longer than 2 years, and regular sexual intercourse with a gynecologically normal partner with no apparent factors of female factor infertility (which was determined at the clinic by biphasic basal body temperature, P evaluation in luteal phase, ultrasound ovary and uterus evaluation, and hysterosalpingogram to study tubal patency).

The specific inclusion criteria were absence of [1] general and endocrinological diseases (studied by clinical examination and routine and hormonal laboratory tests), [2] present or previous cryptorchidism, [3] genital infections or genital tract obstructions (evaluated by sperm culture, urethral swab chlamydia test, and biochemical study of seminal plasma), [4] varicocele and testicular hypotrophy (screened by ultrasound and Doppler color flow), or [5] antisperm antibodies (tested both in sera and bound to the sperm surface) (36). Patients were requested to follow a standard diet to avoid effects due to variable L-carnitine intake in food. None of the patients suffered from L-carnitine metabolism deficiency.

The seminological inclusion criteria were normal rheological characteristics (appearance, consistency, and liquefaction), volume and pH in the normal range, sperm concentration $10–20 \times 10^6$/mL, total motility 10%–30%, forward motility $<15\%$, atypical forms $<70\%$, semen leukocytes $<1 \times 10^6$/mL, and sperm velocity and linearity as evaluated by CASA of 10–30 μ/s and <4, respectively. These upper and lower limits allow the inclusion of cases of mild oligoasthenospermia and were chosen on the basis of possible L-carnitine action on sperm energetic metabolism and on protection against oxidative damage. The lower limits allowed the exclusion of cases of very severe OAT related to irreversible primary or secondary testicular damage, which would inhibit observance of positive or negative effects on seminal variables, as this was, to our knowledge, the first placebo-controlled study of carnitine use in OAT therapy.

For inclusion in the trial, patients had to meet the above seminological inclusion criteria at the time of the first control ($T - 2$), maintain sperm variables within this range for the further two washout controls ($T - 1$ and T_0), and show no statistically significant differences in the three evaluations before treatment ($T - 2$, $T - 1$, T_0).

Statistical Analysis

Means and SD were calculated on all clinical and seminal variables at each time control. An analysis of variance for repeated measures was then performed on the initial three washout semen analyses to test differences between these controls. Student's *t*-test was used to evaluate the homogeneity between placebo and therapy patient groups at baseline. Carryover effect was tested by means of Grizzle's method (37), performed using the Wilcoxon rank-sum test ($\alpha = 0.05$) with Patient (code), Sequence (therapy/placebo or placebo/therapy), Control (time), and Treatment (therapy or placebo) as the main factors.

To allow a real comparison of the effects of the placebo and therapy in terms of improvement in sperm variables and reduce the effect of baseline values of each patient, primary and secondary efficacy analyses were performed on the difference (Δ) between the end point and baseline values of each test period. This was done by means of the Wilcoxon rank-sum test for crossover studies ($\alpha = 0.05$), using Treatment (L-carnitine therapy or placebo) and Control as the main factors. The Δ value was obtained by the differences in sperm variables for each test period (i.e., [variable at $(T + 2)$ $-$ variable at T_0] and [variable at $(T + 6)$ $-$ variable at $T + 4$], respectively). This was performed on both raw data (e.g., motility percentages) and absolute values in terms of millions of spermatozoa/mL and spermatozoa/ejaculate, which were obtained by multiplying the sperm concentration/mL and spermatozoa/ejaculate by the percent of the total and forward sperm motility. The latter values were used for the diagrams.

To exclude transient decrease in semen quality during washout periods followed by a too sudden improvement, independent of treatment, in the following observation periods, we evaluated the existence of similar outlier situations. These were seen in only five patients and in the period from

$T - 2$ to $T + 2$. We added the following exclusion criteria: further evaluations were performed after exclusion from the statistical analysis of those patients with both a high decrease from $T - 2$ and T_0 (from 30% to 10%) and a response in terms of improvement of sperm motility from T_0 to $T + 2$ greater than 30%.

Efficacy analyses in terms of Δ of absolute number of motile spermatozoa were also performed on subgroups with more critical values of forward motile sperm per ejaculate and per milliliter (<5 and $<2 \times 10^6$, respectively).

The Wilcoxon rank-sum test was also used to evaluate the significant differences of the medians observed in the first period of treatment, comparing those patients who received L-carnitine with those who received placebo. This was done to further analyze possible questions related to the crossover and the length of the washout period between the two treatments.

Finally, the difference between L-carnitine therapy and placebo period in terms of the number of those patients with an improvement in semen variables was tested by means of Fisher's exact test. Fisher's exact test was also used to study the relationship in terms of improvements between seminal free L-carnitine concentration and sperm parameter variations.

RESULTS

Of the 100 patients included, 86 completed the study. Eight pregnancies were achieved during the observation period. Evaluation of female partner menstrual history showed that all pregnancies were achieved during the L-carnitine therapy period (six during the first period of therapy with L-carnitine and two during the second in patients first undergoing treatment with placebo). Of the 14 patients not completing the study, four decided to undergo assisted reproduction (two during therapy and two during placebo treatment), six did not return for the second period of treatment (three after a period of therapy and three after placebo treatment), and four of the eight patients inducing pregnancy during the study decided to stop treatment (all after a therapy period).

Table 1 reports values for semen volume, sperm concentration, motility, and morphology over the pretreatment period. The three semen analyses conducted before treatment did not show statistically significant differences in the analysis of variance for repeated measures conducted on the whole patient population (3×100 analyses). Values measured at the beginning of the first treatment (T_0) were therefore acceptable as the baseline for further comparisons.

The patient groups showed no differences at T_0 between therapy or placebo cycle in semen parameters. Furthermore, the sequence of treatment (carryover analysis) was found to be not significant for sperm variables. In particular, the analysis for total and forward motility gave results of $P =$ .461 and .526, respectively. This allowed use of both sequences in the subsequent analysis. As each patient was treated with both the therapy and placebo, there were 172 treatment periods to compare.

TABLE 1

Analysis of variance for repeated measures on the three washout semen measures of all selected patients means ± SD and *P* values).

	$T - 2$	$T - 1$	T_0	P
Semen volume (ml)	3.28 ± 1.56	3.21 ± 1.50	3.25 ± 1.58	.945
Sperm concentration ($n \times 10^6$/mL)	15.88 ± 3.17	16.04 ± 4.19	16.17 ± 4.66	.890
Total motility (%)	25.35 ± 5.51	24.14 ± 5.0	25.51 ± 5.18	.128
Forward motility (%)	12.53 ± 3.30	11.67 ± 3.50	12.58 ± 3.74	.126
Atypical forms (%)	68.57 ± 2.97	68.06 ± 3.07	68.17 ± 2.68	.441

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Five patients at T_0 demonstrated the lower borderline total motility value (i.e., 10%), with a decrease during the washout period (from 30% at $T - 2$ to 10% at T_0) and showed a great difference (i.e., >30%) between the control T_0 and $T + 2$. These variations in sperm motility appear to be independent of the treatment: three of these patients received placebo and two received L-carnitine in the first period. Both the first and second treatment periods of these patients were excluded from further analyses. Ten therapy/placebo cycles were thus excluded, so the total number of therapy/placebo cycles used in the analysis was 162.

An improvement in semen quality was also observed during treatment with placebo. Statistical efficacy analysis comparing the Δ of sperm values after and before L-carnitine treatment and after and before placebo treatment allowed not only exclusion of baseline value interference, but also observation of the real differences between therapy and placebo results.

A first analysis of differences (Δ) observed between the raw data of percent of total and forward sperm motility, tested by means of the Wilcoxon test both on crossover and on the first period alone of all 172 therapy/placebo cycles, was not statistically significant, although the improvement in total and forward sperm motility was higher in the therapy than in the placebo period.

However, statistically significant differences were observed in total and forward motility percentages ($P = .04$ and $P = .05$, respectively) using the same analysis when the five borderline and outlier patients were excluded from the statistical analysis (Table 2). Similarly, statistically significant positive results of a major increase during L-carnitine therapy compared with placebo were observed for sperm concentration ($P = .01$) and sperm linearity evaluated by CASA ($P = .03$) by excluding the above reported five patients (Table 2).

TABLE 2

Variation in sperm concentration (n × 10^6) in total and forward sperm motility (%) and linearity (index) during treatments (placebo/therapy or vice versa) by excluding outlier data of five patients (Δ and *P* value of Wilcoxon tests of 162 therapy/placebo cycles).

	Period 1 (from T_0 to $T + 2$)				Period 2 (from $T + 4$ to $T + 6$)			
	Δ [($T + 2$) − T_0]	Δ [($T + 2$) − T_0]	Δ [($T + 2$) − T_0]	Δ [($T + 2$) − T_0]	Δ [($T + 6$) − ($T + 4$)]	Δ [($T + 6$) − ($T + 4$)]	Δ [($T + 6$) − ($T + 4$)]	Δ [($T + 6$) − ($T + 4$)]
Treatment	Total sperm motility	Forward sperm motility	Sperm concentration	Sperm linearity	Total sperm motility	Forward sperm motility	Sperm concentration	Sperm linearity
L-carnitine	11.0[a]	16.4[b]	9.0[c]	0.6[d]	3.4[a]	4.5[b]	3.7[c]	0.2[d]
Placebo	8.8[a]	13.9[b]	5.3[c]	0.4[d]	−0.1[a]	0.7[b]	−0.7[c]	−0.2[d]

[a] $P = .04$, Wilcoxon test for crossover designs.
[b] $P = .05$, Wilcoxon test for crossover designs.
[c] $P = .01$, Wilcoxon test for crossover designs.
[d] $P = .03$, Wilcoxon test for crossover designs.

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No statistically significant variation in 172 or 162 cycles was seen in semen volume, sperm velocity analyzed by CASA, α-glycosidase concentration, LPOp, or sperm morphology, even though, for sperm velocity, a greater improvement was evident during the L-carnitine therapy period than during the placebo period.

Figures 1 and 2 show the increase in total motile spermatozoa/mL and forward motile spermatozoa/mL obtained by using absolute values expressed in millions of motile spermatozoa present in the ejaculate and their Δ-values, thus eliminating possible interferences due to spontaneous variation in semen volume. The Wilcoxon test on these differences was again highly significant ($P = .008$ and .006, respectively).

The increase evaluated by Δ of forward motile spermatozoa during the therapy period was more significant in the most critical patients, i.e., in those patients with an initial value of $<5 \times 10^6$ forward motile sperm/ejaculate (55 patients) and above all in those with $<2 \times 10^6$ forward motile sperm/mL (71 patients).

FIGURE 1

Diagram of difference (Δ) in absolute values expressed in millions of total motile sperm/mL from the beginning (base) to the end of the L-carnitine therapy (line with diamonds) and placebo (line with circles) cycles. Values are mean ± SD ($P = .008$).

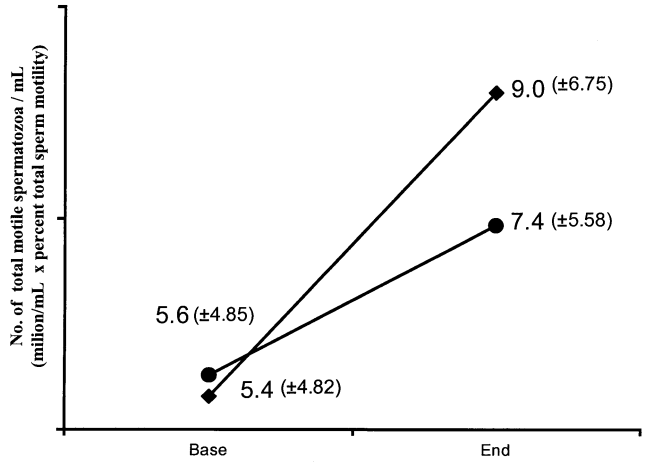

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FIGURE 2

Diagram of difference (Δ) in absolute values expressed in millions of forward motile sperm/mL from the beginning (base) to the end of the L-carnitine therapy (line with diamonds) and placebo (line with circles) cycles. Values are mean ± SD ($P = .006$).

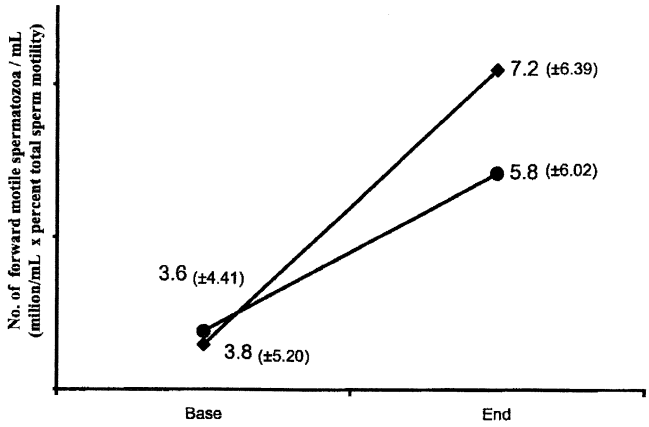

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TABLE 3

Variation in forward motile spermatozoa during treatments (placebo/therapy or vice versa) for the 55 patients with $<5 \times 10^6$ forward motile sperm/ejaculate (means ± SD of the absolute number of forward motile spermatozoa in millions, Δ and *P* values of Wilcoxon tests).

	Period 1 (from T_0 to $T + 2$)			Period 2 (from $T + 4$ to $T + 6$)		
Treatment	T_0	$T + 2$	Δ	$T + 4$	$T + 6$	Δ
L-carnitine	2.9 ± 1.2	14.1 ± 11.0	11.2	2.0 ± 1.7	17.4 ± 28.0	15.4
Placebo	3.3 ± 1.1	10.2 ± 7.3	6.9	2.3 ± 1.4	6.2 ± 7.8	3.9

$P = .03$, Wilcoxon test for crossover designs.

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As shown in Tables 3 and 4, there was a significant difference between the L-carnitine therapy and placebo periods in the absolute number of forward motile spermatozoa in millions ($P =$. 03 and $P = .02$, respectively) in these subgroups. Fisher's exact test on the number of patients showing an improvement during therapy with the number of those showing improvements during the placebo cycle gave results of $P = .04$ and .004, respectively, for patients with $<5 \times 10^6$ and forward motile sperm/ejaculate and $<2 \times 10^6$ forward motile sperm/mL.

Finally, even if carnitine concentration in semen did not show significant variation during L-carnitine therapy, in patients showing an improvement in sperm concentration or in forward or total sperm motility, Fisher's exact test showed a significant relationship between these sperm measures and semen carnitine ($P<.0001$, $P = .014$ and .045, respectively).

DISCUSSION

In recent years, many methods of assisted reproduction have been proposed as a possible solution for "male factor" infertility. These techniques, rather than being a deathblow for andrology, have acted as one of the major impulses for research into sperm function. However, they have also acted as a brake on the development of new strategies for male factor infertility therapy.

TABLE 4

Variation in forward motile spermatozoa during treatments (placebo/therapy or vice versa) for the 71 patients with $<2 \times 10^6$ forward motile sperm/mL (means ± SD of the absolute number of forward motile spermatozoa in millions, Δ and *P* values of Wilcoxon tests).

	Period 1 (from T_0 to $T + 2$)			Period 2 (from $T + 4$ to $T + 6$)		
Treatment	T_0	$T + 2$	Δ	$T + 4$	$T + 6$	Δ
L-carnitine	1.5 ± 0.4	6.2 ± 3.9	4.7	1.0 ± 0.6	5.5 ± 8.2	4.5
Placebo	1.4 ± 0.4	5.0 ± 4.0	3.6	1.0 ± 0.7	2.4 ± 2.8	1.4

$P = .02$, Wilcoxon test for crossover designs.

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Controlled studies in this area share common problems and choices with all clinical trials but also have some special difficulties: case selection criteria, patient acceptance of the placebo period, variables to be analyzed, sperm parameters (having spontaneous variability), sperm function tests (not yet sufficiently standardized), spontaneous pregnancy rate (subject to female contribution), and in vitro fertilization rate (too high to be able to observe significant variations).

Unfortunately, for these reasons many drugs are used in the treatment of male factor infertility without any rationale: such therapies are often prescribed sequentially without any positive effect, and any imagined improvements in semen parameters are without a real basis and may in fact be caused by natural fluctuations in semen quality. Some of these therapies were also proposed in the past in the international literature and were the subject of a wide body of criticism (38). The claimed purpose is to amplify spermatogenesis, boost the highest quality sperm populations, and act on the sperm maturation and energetic metabolism and on the testicular-epididymal microenvironment.

Among these proposed actions, postgonad maturation could be a potentially rational and interesting target especially as it occurs mainly in the epididymal fluid where spermatozoa are far from the complex and still only partially understood intratesticular hormonal network. In the epididymis, the epithelium removes some testicular factors, takes up material from the blood, and produces specific compounds, all useful for sperm maturation and motility. Among these, in the mammalian epididymis, the free L-carnitine is taken up from the blood plasma, transported into the epididymal fluid and into the spermatozoa, and accumulated as both free and acetylated L-carnitine. This small, quaternary amine-free L-carnitine is one of the most concentrated water-soluble polar substances present at the epididymal level (hundreds of times more concentrated than in blood).

Free L-carnitine (3-hydroxy-4-N-trimethylaminobutyric acid) was first isolated from bovine muscle in 1905, and its structure was definitively established in 1927 (26). It is mainly known for its biological importance in mitochondrial beta-oxidation of long-chain fatty acids, as demonstrated by Fritz in 1963. Before entering the mitochondria, fatty acids must be activated, i.e., they must bind to the CoA to form acyl-CoA. Long-chain molecules of acyl-CoA are not able to cross the internal mitochondrial membrane so they need a specific enzymatic shuttle system. After the transport of the acyl into the mitochondria, acyl carnitine transfers the acyl to the mitochondrial CoA and exits as free carnitine to start a new transport cycle (26).

Carnitine also acts in the cell membrane as an "anti-aging" substance, protecting against damage induced by free oxygen radicals. It prevents protein oxidation and pyruvate and lactate oxidative damage. In humans, 75% of carnitine derives from diet in the same way as other water-soluble substances, while 25% is synthesized from lysine and methionine, although the enzyme that catalyses the hydroxylation of the 4-butirrobetain in L-carnitine, 4-butirrobetain hydroxylase, is present in few tissues (27).

From all the above knowledge on the action of carnitine on cellular metabolism, and from results obtained in a previous (uncontrolled) multicenter study (29) and recently confirmed in selected andrological pathologies (30), we selected L-carnitine as possibly active on parameters relating to male factor infertility and conducted the present controlled study (double blind vs. placebo, crossover). We used a 2-month therapy/placebo period to focus attention on the effect directly mediated by carnitines on spermatozoa or late spermatogenesis phases. The number of selected patients, taking into account the strict criteria under which they were selected and the number of seminal analyses conducted per patient in a controlled trial, carries some weight with regard to both the results obtained and all the well-known difficulties involved in performing such rigorous studies and interpreting their results.

We tried to reduce possible bias in the general scheme of the study. The washout period with three semen analyses before the start of therapy allowed evaluation of the therapeutic effect while minimizing the possible effect of spontaneous variations in seminal characteristics (31). The 2-month washout period between administration of the therapy and placebo (or vice versa) avoided incorrect attribution of their effects. However, to eliminate possible queries regarding the validity of the crossover design and the duration of the washout period between the two treatments, statistical analysis was carried out by the Wilcoxon test for the crossover periods and also separately for the first period.

First, we observed that there were improvements in the variables analyzed even during administration of the placebo that could be only in part due to the statistical phenomenon of regression to the mean. Additionally, the therapy gave a stronger positive effect in the first period of administration than in the second. These results strongly confirm that an important psychological element exists even in infertility, related to the sensation of being treated, and the continuous availability of medical staff and counseling. This is particularly evident in the first phases of therapy.

Second, we observed that seminal concentration of L-carnitine did not show significant improvement during therapy. However, it is well known in other fields of medicine that the activity of carnitines is not related to their concentration in serum or biological fluids (26, 28). As their action is at an intracellular level, it may be necessary to evaluate possible molecular and metabolic modifications induced by the therapy. This is particularly true for semen, where there is a massive concentration of carnitine. We were therefore not surprised to find that carnitine concentration did not show significant variation. A peak of serum and semen concentration may occur at some time after oral administration, but studies dedicated to pharmacokinetics also failed to show semen concentration improvement induced by therapy (26).

However, even though the increase in concentration of L-carnitine in semen was not statistically significant because the initial seminal concentration is so high as to prevent this, there was a significant improvement in seminal parameters of those taking carnitine, compared with those taking the placebo; this improvement is statistically related to the variation in semen carnitine concentration. This means that even if the variation in seminal carnitine is not at a significantly statistical level, an improvement in sperm parameters corresponds to a slight improvement in seminal carnitine. In particular, the highly significant relationship with sperm concentration ($P<.0001$) observed by Fisher's test could indicate that an increase occurs only in intracellular sperm carnitine, but this is too slight to influence the seminal concentration.

Furthermore, the absence of a significant increase in seminal α-glycosidase concentration appears to confirm an absence of an overall improvement in epididymal function. Additionally, the nonsignificant modifications in LPOp values showed no direct effect in sperm membrane fatty acid composition. Antioxidant activity, using a relatively short therapy period of 2 months, may have a more important effect on the microenvironment than on the cell membrane structure and capacity to counteract reactive oxygen species.

The most expected effect from a prognostic point of view of the patient's fertility was that on impaired sperm motility. The reason for this effect must be the metabolic energetic action of L-carnitine and its activity as an anti-oxidant compound (39). Using the sperm motility/mL and sperm motility/ejaculate percentage data of all 86 patients, the difference in improvement between therapy and placebo, although present, was not statistically significant.

The improvement becomes statistically significant when the outlier data of those five patients who showed a spontaneous decrease in sperm motility during the washout period were eliminated. This decrease was too great to exclude a transient, although nonsymptomatic, pathology followed by a too sudden and large improvement in the first treatment period that appears independent of that same treatment. Furthermore, results become highly statistically significant by using absolute data of total number of spermatozoa with motility or forward motility present in the ejaculate. This last analysis allows exclusion of the confounding effect of semen volume variation, which is not dependent on the therapy. The significant increase in sperm linearity and the nonsig-

nificant variation in sperm velocity seem to indicate a selective effect on the qualitative mechanisms of sperm kinetics.

The results on sperm motility are made even more interesting by the fact that its greatest effect is found in the most critical cases, that is, those with the lowest initial forward motile sperm concentration. The effects on these subgroups were very interesting from both a speculative and clinical point of view. First, we can hypothesize that even with a reduced spermatogenesis, biochemical deficiency in the energetic metabolism of mitochondria in such patients could be corrected by a high dose of intracellular carnitine. Second, these results, showing a possible action of L-carnitine even in cases of very severe OAT (e.g., sperm motility $<10\%$)—not included in the present study—could be an indication for future studies of patients with very low semen values.

While the effect on sperm motility was suggested by the metabolic action of the carnitine, the effect observed on sperm concentration was unexpected. The characteristics of carnitine would not lead one to expect a direct effect on the first phases of spermatogenesis, but rather on post-testicular sperm maturation. Moreover, as the therapy period chosen (2 months) was not long enough to affect a complete spermatogenic cycle (approximately 75 days), it was not possible to detect an effect on the complete spermatogenic cycle. This effect on sperm concentration may be due to an unknown effect in Sertoli cell–spermatogenic line interaction, to an action on the postmeiotic phases of spermatogenesis (for example, on the chromatin stability or mitochondrial function of spermatocytes or spermatids), or to an improvement in homeostasis and the quality of the epididymal microenvironment, reducing gamete phagocytosis at this level while increasing ejaculated spermatozoa.

The lack of effects on sperm morphology, considered as one of the most sensitive indices of the efficacy of spermatogenesis, seems to confirm the hypothesis of a post-testicular effect.

Finally, even though pregnancy is not considered the principal end point for this controlled study because of the many possible interferences, the eight pregnancies, with spontaneous in vivo fertilization all obtained during the L-carnitine therapy period, from a group of patients with long-term infertility could suggest that carnitines may also lead to an improvement in sperm function and fertilization capacity. This could be a further indication for future studies including patients undergoing assisted reproduction.

To confirm all the above, it will also be necessary to study in vitro the effect of carnitine on the metabolism of the male gamete with molecular and cellular studies and to carry out longer and multicenter controlled trials.

In conclusion, a general recommendation is that both clinical reproduction specialists and general practitioners should have andrological training to enable them to recognize and treat pathologies associated with a decrease in male fertility, e.g., cryptorchidism, varicocele, sexually transmitted diseases, environmental and work factors, and lifestyle (40–43). When andrological pathologies are not evident, only those therapies that have been subjected to controlled studies should be used to treat OAT (9). L-carnitine therapy, submitted to such a study, showed some interesting positive effects in increasing semen parameters that merit further investigation.

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