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Cinnoxicam and L-Carnitine/Acetyl-L-Carnitine Treatment for Idiopathic and Varicocele-Associated Oligoasthenospermia

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ABSTRACT: The objective of this study was to detect a therapy for idiopathic and varicocele-associated oligoasthenospermia (OAT). Idiopathic and varicocele OAT patients were randomized into 3 groups. Each group was composed of varying degrees of left varicoceles (graded into 5 grades with echo-color Doppler) and of idiopathic OATs. Group 1 used a placebo, group 2 used oral L-carnitine (2 g/d) + acetyl-L-carnitine (1 g/d), group 3 used L-carnitine/acetyl-L-carnitine + 1 × 30-mg cinnoxicam suppository every 4 days. Drugs were administered for 6 months. The groups were composed as follows: group 1, 71 varicoceles and 47 idiopathic OATs; group 2, 62 varicoceles and 39 idiopathic OATs; group 3, 62 varicoceles and 44 idiopathic OATs. Sperm concentration, motility, and morphology before during and after treatments were assessed. Pregnancy rates and side effects were recorded. Group 1 did not have modified sperm patterns during treatment. Group 2 had significantly increased sperm patterns at 3 and 6 months into therapy in idiopathic patients and in patients with grades I, II, and III varicocele, but not in grades IV and V. Group 3 had significantly increased sperm parameters in all patients, with the exception of grade V varicocele. Group 3 sperm patterns proved significantly higher during therapy than group 2. All sperm patterns fell to baseline after therapy suspension. Minor side effects occurred. Pregnancy rates were 1.7% (group 1), 21.8% (group 2), and 38.0% (group 3) ($P < .01$). L-Carnitine/acetyl-L-carnitine + cinnoxicam suppositories proved a reliable treatment for low-grade varicoceles and idiopathic OATs.

Key words: Male infertility, idiopathic oligoasthenospermia, varicocele, L-carnitine, acetyl-L-carnitine, cinnoxicam.

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About 30% of oligoasthenoteratospermic (OAT) infertile men are still classified as ''idiopathic,'' in that no ''etiological'' treatment has been found; therefore ''empirical'' drugs have been used. Idiopathic OAT males were regarded as impervious to any therapy until a few years ago (Bonanomi et al, 2002). Only a few and very recent prospective trials increased sperm count and spontaneous pregnancies in idiopathic OAT infertile men. Adamapoulos et al. (2003) used tamoxifen and testosterone, Wong et al (2002) used zinc sulfate and folic acid. Folliculostimulating hormone (FSH) increased sperm production in idiopathic infertile patients with normal inhibin B plasma levels and a testicle cytological picture of hypospermatogenesis (Foresta et al, 2002). Fine needle aspiration (FNA) was used to classify the patients, but open testicular biopsy (Devroey et al, 1995) and FNA (Lewin et al, 1999) might affect spermatogenesis and sperm count. Lenzi et al (2003) successfully used L-carnitine in idiopathic infertile males. Vicari indirectly confirmed his results demonstrating that L-carnitine + acetyl-L-carnitine increased sperm count in patients with echographic features of genital inflammation but with white blood cell sperm concentration of less than 10^6/mL and negative sperm culture (Vicari and Calogero, 2001).

A significant but clinically undetectable venous reflux (ie, subclinical varicocele) was found by scrotal echo-color Doppler at cord level in many OAT patients (Gattuccio et al, 2000). However, OAT associated with subclinical varicocele remains controversial: a moderate increase in sperm count was found after surgery (Yamamoto et al, 1996), but current evidence indicates that varicocele size does matter and that large varicoceles are more likely to improve with surgery. Appropriate medical treatment is warranted because surgery for subclinical varicoceles is not recommended (Jarow, 2001); however, no medical treatment could be successfully used until now.

Our team has demonstrated that intermittent administration of cinnoxicam (a nonsteroidal anti-inflammatory drug [NSAID]) suppositories improved sperm concentration, motility, and morphology in echo-color Doppler grade III varicoceles, but not in grades IV and V (Cavallini et al, 2003). In this study, we looked to see whether cinnoxicam improved the efficacy of carnitines in idiopathic and varicocele-associated OAT patients.

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Patients and Methods

The study was authorized by the Studi di Medicina della Riproduzione (SISMER) institutional review board. Patient recruitment began on January 2, 1999, and finished on December 1, 2002. Written informed consent was obtained from each patient.

All OAT patients with deficiencies in all sperm patterns (sperm concentration < 20 000 000/mL, class A motility < 25%, typical forms < 30%) whose chief complaint was primary couple infertility longer than 12 months and regular intercourse (World Health Organization [WHO], 1999) were candidates for inclusion in the study. Eligible patients were those with normal sperm appearance, consistency, liquefaction, volume, and pH (WHO, 1999). A female partner free of any identifiable factor of infertility was required for eligibility. Female infertility factors were determined by the SISMER clinic with a medical history collection, an objective examination, a biphasic body temperature recording, a progesterone evaluation in luteal phase, an ultrasound of the uterus and of the ovaries, and a hysterosalpingogram to study tubal patency (Lenzi et al, 2003).

They were not admitted into the study if any of the following criteria were present: azoospermia (14 patients); seminal white blood cell concentration more than 1 000 000/mL, positive seminal cultural analysis, or positive urethral swab chlamydia test (27 patients); oligospermia less than 5 000 000/mL (11 patients); hormonal alterations (6 patients); age more than 40 years (10 patients); presence of anti-sperm antibodies (tested both in sera and bound to sperm surface, 12 patients; Lenzi et al, 2003); drug, tobacco, or alcohol abuse (18 patients); ongoing medical treatments (gonadotropins, anabolic steroids, cancer chemotherapy, NSAIDs; 6 patients); presence of hydrocele (4 patients); diabetes (2 patients); hypertension (4 patients); x-ray exposure in the previous 8 months (3 patients); peptic ulcer (1 patient); unexplained gastric pain (7 patients); previous hypersensitivity to NSAIDs or carnitines (0 patients); carnitine metabolism deficiency (0 patients); bilateral varicocele found with echo-color Doppler scanning or objective examination (29 patients); or prostate abnormalities found with digital rectal exploration or suprapubic ecography (19 cases). Prostate abnormalities were considered as volume more than 20 cm^3, tenderness, modifications of consistency found with digital rectal exploration and modifications of echogenicity found with suprapupic echoscan, or total serum prostatic-specific antigen higher than 4 μg/mL (Adamapoulos et al, 2003). Further exclusion criteria were previous or concurrent testicular pathology (torsion, undescended, orchi-epididymitis, surgery, trauma, or neoplasm) or testicle echographic abnormalities (intended as focal or diffuse change of testicle structure or of volume). Left testicular atrophy associated with clinical grade III or duplex grade IV and grade V varicoceles were not considered exclusion criteria (see Patient Sample section).

Patient Sample

This study considered 380 patients; our results include 325 patients (age range 27–40, mean age 34 years) because 55 dropped out: 30 patients refused to participate in a study where a placebo might be administered, 1 divorced, 1 reported a severe car crash, 6 preferred assisted reproduction techniques, 5 naturally conceived, and 10 patients made mistakes in drug consumption. All patients underwent a medical history collection, sexological counseling session(s), an objective examination, an anti-sperm antibody detection, a bilateral scrotal echo-color Doppler scanning and a testicular volume calculation (cm^3) with the 3-diameters technique (Gattuccio et al, 2000), a dosage of serum (PSA, FSH, luteinizing hormone [LH], prolactin, total and free testosterone), and a suprapubic prostatic echoscan. Three samples of WHO 1999 sperm analysis were obtained from each patient (WHO, 1999). Semen culture analyses and chlamydia urethral swabs were performed on each patient.

Varicocele is intended as a venous reflux detectable with echo-color Doppler longer than 2 seconds at the level of the pampiniform plexus with or without the Valsalva maneuver. Varicocele was detected in 203 patients and classified by bilateral echo-color Doppler scanning according to the severity of venous reflux (Gattuccio et al, 2000): venous reflux with Valsalva maneuver limited to the cranial portion of the cord (grade 1), reflux with Valsalva until the upper pole of the testicle (grade 2), reflux with Valsalva until the lower pole of the testicle (grade 3), reflux in basal conditions increased by Valsalva (grade 4), reflux in basal conditions which does not increase with Valsalva (grade 5). Approximately duplex grades 1 and 2 correspond to subclinical varicoceles; duplex grades 3, 4, and 5 duplex varicoceles correspond to clinical grade 1, 2, and 3, respectively (Dubin and Amelar, 1970; Gattuccio et al, 2000). Distribution of patients according duplex and clinical classification is reported in Table 1.

The testicular volume calculation revealed left testicular atrophy (intended as testicular volume ≥ 20% lower than the counterpart) in 67 varicoceles (Gattuccio et al, 2000).

Patients (123) were classified as idiopathic OAT because any known or demonstrable causes of dyspermia and of infertility could be found in the course of the above assessments (Foresta et al, 2002; Adamapoulos et al, 2003; Lenzi et al, 2003). Each varicocele grade or idiopathic OAT was considered 1 experimental unit.

Patient Randomization

The patients were asked to follow a standard diet (2000–2600 kcal in 3–5 meals: proteins 15%, sugars 52%, lipids 33% [Gatti, 1980]) in order to avoid the effects of variable carnitine intake. The patients were randomized in 3 groups. Starch tablets (500 mg) and commercial glycerine suppositories (Glicerolo supposte 2500 mg; Carlo Erba, Milano, Italy) were used as placebos. Three groups of treatment were used. The first group used 1 × 500-mg starch tablet twice daily and 1 glycerine suppository every 4 days. The second group used oral L-carnitine 1 × 2 g/d (Carnitene, Sigma-Tau, Pomezia, Italy) + oral acetyl-L-carnitine 500 × 2 mg/d (L-carnitine/acetyl-L-carnitine, Zibren, Sigma-Tau, Pomezia, Italy) + 1 glycerine suppository every 4 days. The third group used L-carnitine/acetyl-L-carnitine + 1 × 30-mg cinnoxicam suppository (Sinartrol, Società Produttrice Antibiotici, Milano, Italy) every 4 days. The composition of the treatment groups is presented in Table 1.

Active drug placebos identical in appearance could not be prepared. The following procedures were adopted to ensure blindness (Byington et al, 1985). Independent pharmacists prepared, anonymized, and certified starch tablets; anonymized carnitine and cinnoxicam and glycerine suppository containers; and filled and sealed anonymous color-coded boxes containing drugs, pla-

Table 1. *Epidemiologic data of the patients participating in the study; varicoceles were classified with echo-color Doppler scrotal scanning; parentheses enclose the corresponding clinical classification*

	No. of Patients		
	Group 1	Group 2	Group 3
Experimental units			
Idiopathic oligoasthenoteratospermic males	47	39	44
Grade I varicoceles*	11	9	8
Grade II varicoceles	13 (12 subclinical and 1 clinical grade I varicoceles)	12 (10 subclinical and 2 clinical grade I varicoceles)	10 (8 subclinical and 2 clinical grade I varicoceles)
Grade III varicoceles	20 (17 clinical grade I and 3 clinical grade II varicoceles)	18 (16 clinical grade I and 2 clinical grade II varicoceles)	18 (17 clinical grade I and 1 clinical grade II varicoceles)
Grade IV varicoceles†	17‡	15‡	15§
Grade V varicoceles‖	10	8	11
Presence of testicle atrophy	23	21	23
Patient age (years)			
Mean	34	35	33
Range	29–40	28–39	27–40
Partner age (years)			
Mean	31	32	31
Range	27–39	26–38	25–39
Duration of couple infertility (months, mean ± SD)	15.3 ± 2.6	16.2 ± 3.1	15.9 ± 2.4

* All duplex grade I varicoceles were subclinical.
† All duplex grade IV varicoceles were clinical grade II.
‖ All duplex grade V varicoceles had left testicular atrophy and were clinical grade III.
§ Twelve patients had left testicular atrophy.

cebos, or both; the code was transmitted to the Institutional Review Board (IRB) of SISMER. The color code was disclosed to physicians by pharmacists and by IRB at the end of the research. Written instructions to correctly consume box contents were prepared by the physicians and enclosed by the pharmacists. The pharmacists ensured and certified that patients had correctly consumed the substances, and they informed the physicians without revealing whether the substances were active drugs or placebos. Each box contained the quantity of drugs, placebos, or both necessary for 3 months. The boxes were delivered blindly by nurses on the basis of casual number tables (Snedecor and Cochran, 1987). Drugs were administered for 6 months. All study personnel and participants were blinded to treatment assignment for the duration of the study. Only the SISMER IRB saw unblinded data to alert physicians in the case of major side effects, but this occurrence never happened and no one from the IRB had any contact with study participants. Secretarial personnel not participating in the trial asked nurses, physicians, and patients before periodic data collection and drug delivery whether they were delivering or consuming group 1, group 2, or group 3 treatments. The blinding procedure was considered successful if the percentage of correct answers did not significantly differ in the chi-square test from the theoretical value of 33%. Medical doctors answered correctly in 31% of the cases ($\chi^2 = 1.33$, P not significant), patients in 36.5% ($\chi^2 = 2.030$, P not significant), and nurses in 35.7% ($\chi^2 = 1.098$, P not significant).

Variables Examined

In this study, we tested the hypotheses that carnitines or carnitines + cinnoxicam can improve sperm patterns and natural pregnancy achievement in infertile couples because of idiopathic OAT or OAT associated with varicocele.

The primary end points were side effects, bilateral testicular volume (cm^3), sperm concentration (millions/cm^3), percentage of WHO class A motile sperm and of incidence of typical forms and side effects. The data were collected before, during (at 3 and 6 months), and after (3 months after) therapy. Sperm patterns had been measured on 3 consecutive analyses (WHO, 1999).

The secondary end points were natural pregnancies achieved during the observation period of 9 months and their assumed time of spontaneous fertilization. The pregnancies were recorded monthly and estimated on the basis of the last ovulatory period of the female partner before the first β-human chorionic globulin–positive result (Lenzi et al, 2003).

Data Analyses

Data analyses were carried out according to a pre-established plan.

Sperm patterns and testicular volume were analyzed as follows. Multiple comparisons of paired series of data (ie, data comparison of each experimental unit within an identical group of treatment) used randomized block (1 patient = 1 block) analysis of variance (ANOVA), coupled paired comparisons used randomized block orthogonal comparisons. Multiple comparisons of independent series of data (ie, data comparison of each experimental unit between/among different groups of treatment) used ANOVA, coupled independent comparisons used orthogonal comparisons. When sperm patterns were submitted for analysis, the tests were corrected for the number of replicated sperm

Table 2. *Seminal parameters measured before, during, and after treatment with placebo, carnitines associated with cinnoxicam or carnitines done; data are presented as 1 median and one second to third interquartile; 1 mean and 1 interquartile of 3 samples have been presented for the sake of brevity; each mean and interquartile are the medians of 3 separate replicated estimates of the variable**

	Group 1 (Placebo)				Group 2 (L-carnitine + Acetyl-L-Carnitine)				Group 3 (L-Carnitine + Acetyl-L-Carnitine + Cinnoxicam)			
	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
Grade 1 varicoceles												
Concentration × 10^6/ml												
Median	**11.1**	**12.4**	**12.0**	**10.8**	**12.3**	**17.3**	**18.6**	**11.9**	**10.0**	**30.0**	**31.1**	**10.7**
Interquartile	15.2–8.6	14.7–7.8	15.1–6.9	15.0–6.8	14.4–8.8	23.9–14.1	24.1–15.0	14.0–8.8	14.3–8.9	22.5–40.3	23.6–41.1	13.9–7.9
WHO class A motile sperm, %												
Median	**13.5**	**14.0**	**13.7**	**12.8**	**13.0**	**17.5**	**16.8**	**11.9**	**13.3**	**31.7**	**31.1**	**11.6**
Interquartile	16.6–8.8	15.4–8.0	15.8–7.7	14.9–8.9	14.3–9.1	23.0–12.1	22.1–12.7	13.8–6.3	12.8–8.9	36.3–26.4	34.3–27.2	13.6–5.9
Typical forms, %												
Median	**15.4**	**14.8**	**13.6**	**14.8**	**16.5**	**25.5**	**24.7**	**15.9**	**15.7**	**36.8**	**39.2**	**14.7**
Interquartile	19.1–11.18	18.5–10.8	15.9–10.0	17.1–11.7	20.0–12.8	30.6–19.4	29.9–18.2	18.7–13.9	18.6–11.0	42.1–30.1	45.6–31.4	20.3–9.9
Grade 2 varicoceles												
Concentration × 10^6/ml												
Median	**10.6**	**11.9**	**12.3**	**11.0**	**11.3**	**18.4**	**17.6**	**12.9**	**9.8**	**29.3**	**30.6**	**10.3**
Interquartile	14.0–8.3	15.5–7.6	15.7–6.7	16.8–8.8	14.4–9.6	23.2–13.6	22.6–12.5	14.2–10.0	15.3–7.8	36.4–21.8	38.6–22.0	14.2–7.8
WHO class A motile sperm, %												
Median	**14.7**	**15.1**	**13.7**	**13.0**	**13.1**	**18.1**	**18.3**	**12.1**	**13.4**	**31.6**	**29.8**	**11.0**
Interquartile	18.8–8.6	17.9–8.7	17.4–9.0	17.0–8.0	18.2–8.6	24.0–13.0	24.0–12.1	16.4–6.8	17.9–6.6	40.1–27.3	40.4–27.7	14.1–6.4
Typical forms, %												
Median	**15.0**	**14.7**	**16.3**	**14.7**	**16.5**	**26.5**	**24.3**	**14.6**	**16.0**	**38.0**	**39.2**	**13.0**
Interquartile	18.3–9.4	17.2–8.9	19.2–9.6	18.3–9.3	19.0–8.6	31.6–20.3	30.6–19.4	18.4–10.1	19.0–10.0	45.2–33.6	46.3–32.4	16.1–9.2
Grade 3 varicoceles												
Concentration × 10^6/ml												
Median	**11.4**	**12.3**	**13.0**	**11.4**	**10.0**	**19.3**	**22.0**	**12.1**	**11.4**	**34.6**	**36.2**	**10.3**
Interquartile	14.2–8.7	15.3–8.6	16.0–9.0	16.3–8.5	13.4–7.0	23.4–14.0	22.1–13.6	13.0–7.7	15.2–7.8	41.3–29.2	42.0–30.0	14.6–8.7
WHO class A motile sperm, %												
Median	**12.8**	**13.2**	**13.5**	**11.0**	**15.2**	**16.5**	**17.0**	**14.4**	**13.0**	**34.0**	**35.1**	**12.3**
Interquartile	16.2–8.9	17.3–9.6	18.1–10.0	15.3–7.9	20.0–11.0	21.3–11.0	20.3–11.6	19.7–10.2	16.8–9.0	40.3–29.6	40.7–30.2	15.9–8.6
Typical forms, %												
Median	**17.4**	**16.8**	**16.0**	**15.4**	**15.3**	**25.0**	**24.8**	**14.2**	**16.0**	**34.5**	**36.2**	**14.2**
Interquartile	21.3–12.4	19.9–11.6	19.2–10.3	19.0–9.9	20.9–11.8	29.8–20.6	30.1–21.6	18.7–7.2	20.2–9.9	41.3–28.5	40.6–30.0	19.4–8.7
Grade 4 varicoceles												
Concentration × 10^6/ml												
Median	**11.5**	**11.2**	**10.9**	**9.8**	**12.7**	**12.5**	**12.0**	**12.1**	**10.7**	**19.3**	**18.4**	**11.1**
Interquartile	15.0–9.1	14.7–8.9	15.8–8.6	14.9–9.2	16.1–9.2	16.4–8.6	16.0–8.0	16.1–8.2	14.3–9.8	24.6–14.3	24.7–14.8	14.5–10.1

Table 2. *Continued*

	Group 1 (Placebo)				Group 2 (L-carnitine + Acetyl-L-Carnitine)				Group 3 (L-Carnitine + Acetyl-L-Carnitine + Cinnoxicam)			
	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
WHO class A motile sperm, %												
Median	**14.1**	**13.6**	**12.1**	**15.2**	**15.0**	**13.9**	**16.0**	**15.5**	**14.6**	**23.8**	**25.2**	**12.3**
Interquartile	18.0–10.2	17.9–9.8	16.9–8.6	17.9–9.2	19.2–9.6	18.6–8.9	19.7–9.2	19.7–10.6	19.1–8.6	18.6–8.0	17.9–7.5	20.1–8.1
Typical forms, %												
Median	**15.2**	**16.4**	**15.9**	**16.0**	**14.5**	**16.2**	**15.9**	**13.2**	**15.0**	**28.3**	**26.4**	**13.2**
Interquartile	21.3–8.6	20.6–9.1	20.5–10.0	21.6–9.8	20.7–8.3	20.4–9.1	21.6–10.0	19.1–8.6	19.7–9.6	32.3–21.6	30.4–20.7	18.6–9.3
Grade 5 varicoceles												
Concentration × 10^6/ml												
Median	**11.1**	**12.4**	**11.4**	**13.0**	**12.0**	**11.6**	**11.6**	**12.0**	**12.0**	**11.6**	**11.0**	**10.0**
Interquartile	14.9–8.7	15.2–9.1	16.0–9.1	16.6–9.2	16.3–10.2	15.4–9.6	16.0–8.7	15.6–9.0	16.7–8.6	15.8–7.8	14.9–7.0	14.0–7.3
WHO class A motile sperm, %												
Median	**13.7**	**16.1**	**14.1**	**15.2**	**17.1**	**16.9**	**13.9**	**15.6**	**14.5**	**17.2**	**16.1**	**15.8**
Interquartile	18.6–7.2	19.4–8.8	19.6–8.2	19.2–7.8	19.4–11.6	20.1–12.4	19.2–8.4	20.0–12.8	18.6–9.9	19.4–11.0	19.2–13.6	19.3–11.8
Tyipcal forms, %												
Median	**13.6**	**15.0**	**14.2**	**15.6**	**15.4**	**16.0**	**17.1**	**15.0**	**15.8**	**17.0**	**14.8**	**13.0**
Interquartile	18.6–8.3	19.4–8.6	17.2–9.2	19.2–8.8	20.7–7.6	21.5–8.9	21.3–10.0	20.6–9.2	20.8–10.3	21.3–9.7	20.6–8.2	19.8–8.6
Idiopathic oligoasthenoteratospermic males												
Concentration × 10^6/ml												
Median	**11.6**	**12.3**	**10.9**	**12.7**	**12.1**	**20.9**	**20.6**	**11.1**	**12.0**	**33.2**	**34.0**	**10.6**
Interquartile	15.0–8.7	16.0–9.1	15.1–9.0	14.8–8.6	15.6–9.0	25.6–14.8	24.9–15.1	14.9–9.5	14.9–8.6	40.8–26.3	41.0–27.1	14.7–8.6
WHO class A motile sperm, %												
Median	**13.3**	**14.0**	**13.2**	**14.0**	**11.0**	**22.3**	**23.6**	**12.7**	**11.9**	**36.7**	**39.4**	**12.3**
Interquartile	16.0–8.6	17.4–5.1	18.6–9.0	19.2–9.6	14.3–5.6	28.4–15.2	28.9–16.0	14.7–8.4	15.2–8.4	42.2–30.1	45.2–33.2	15.6–6.9
Typical forms, %												
Median	**19.1**	**18.7**	**15.3**	**16.6**	**16.6**	**26.5**	**27.3**	**15.9**	**15.9**	**38.8**	**40.0**	**14.3**
Interquartile	24.1–15.6	23.0–14.0	22.0–12.1	21.6–10.7	21.7–12.3	31.3–21.6	32.0–22.6	20.0–11.6	18.9–11.7	43.2–31.3	44.2–33.1	18.6–9.7

* T0 indicates measures before therapy; T1, 3 months after the start of therapy; T2, 6 months after the start of therapy; and T3, 3 months after therapy suspension.

Table 3. *Bilateral testicular volume before, during, and after therapy; data are means ± SD; results were not significant*

Group	Varicocele Grade	Testicular Volume, cm^3				
		Before Therapy	3 Months After Therapy	6 Months After Therapy	3 Months After Therapy Suspension	*F*
1	I	31.4 ± 1.9	32.0 ± 2.0	31.7 ± 1.9	32.3 ± 2.1	1.1
	II	30.9 ± 2.0	31.3 ± 1.7	30.8 ± 1.3	31.4 ± 1.8	<1
	III	30.9 ± 2.6	31.4 ± 3.0	31.2 ± 2.9	31.0 ± 2.9	1.2
	IV	25.2 ± 1.7	24.9 ± 1.6	25.6 ± 1.9	24.9 ± 1.7	<1
	V	23.1 ± 2.1	23.4 ± 2.2	23.4 ± 2.0	23.0 ± 1.9	<1
	Idiopathic males	31.9 ± 1.7	32.1 ± 1.8	32.0 ± 1.9	31.9 ± 1.7	1.1
2	I	31.7 ± 2.0	31.6 ± 1.9	32.0 ± 1.9	31.8 ± 1.7	<1
	II	31.8 ± 2.0	31.6 ± 1.7	32.1 ± 1.9	31.8 ± 1.5	<1
	III	31.6 ± 1.9	31.5 ± 1.7	32.0 ± 1.9	31.6 ± 1.3	1.1
	IV	24.0 ± 2.0	23.6 ± 1.8	24.4 ± 2.0	24.0 ± 1.8	<1
	V	22.0 ± 1.2	21.6 ± 1.3	22.0 ± 1.2	22.2 ± 1.0	1.2
	Idiopathic males	31.6 ± 1.9	31.5 ± 1.6	31.9 ± 1.9	31.6 ± 1.3	1.2
3	I	31.3 ± 1.7	31.2 ± 1.5	31.7 ± 1.9	31.3 ± 0.9	1.9
	II	31.7 ± 1.7	31.7 ± 1.7	32.2 ± 1.9	31.6 ± 0.9	<1
	III	31.5 ± 1.7	31.5 ± 1.6	32.0 ± 1.9	31.5 ± 0.9	<1
	IV	24.0 ± 1.9	23.5 ± 1.7	24.1 ± 1.8	23.8 ± 1.9	1.2
	V	22.8 ± 1.3	22.8 ± 0.4	23.2 ± 0.8	22.8 ± 0.8	1.1
	Idiopathic males	31.5 ± 1.6	31.4 ± 1.5	31.9 ± 1.8	31.4 ± 0.9	1.2

analyses. Testicular volume used natural data, sperm concentration used log-transformed data, and motility and morphology used angular-transformed ($\sin^{-1}\sqrt{P/100}$) data (Snedecor and Cochran, 1987).

Percent incidence of side effects and of naturally achieved pregnancies were compared with the chi-square test among groups, between groups, or both (Snedecor and Cochran, 1987).

Results

Sperm concentration, motility, and morphology data are presented in Table 2, which is organized into 6 parts: 1 for each experimental unit. No significant difference emerged among the baseline values of concentration, motility, and morphology of the three groups in the grade I varicocele results. Group 1 showed no changes in sperm concentration, motility, and morphology with therapy. Groups 2 and 3 had significantly increased sperm concentration motility and morphology at 3 and 6 months in the course of therapy; no significant difference occurred between the data of 3 and 6 months within each treatment group. Sperm concentration, motility, and morphology of group 3 in the course of treatment were significantly higher than the correspondent values of group 2. Three months after drug suspension, concentration, motility, and morphology data fell back to the baseline. For grade II varicoceles, grade III varicoceles, and idiopathic OATs, results were not significantly different from grade I varicoceles. For grade IV varicocele data, no significant difference emerged from the baseline data of groups 1, 2, and 3. Groups 1 and 2 did not have significantly increased sperm concentration, motility, and morphology at 3 and 6 months. Group 3 had significantly increased sperm concentration, motility, and morphology at 3 and 6 months, but to a significantly lesser extent than in idiopathic OATs and than in grade I, II, and III varicoceles. Three months after drug suspension, sperm concentration, motility, and morphology data fell back to the baseline. For grade V varicocele data, no significant difference in sperm concentration, motility, and morphology from the baseline emerged in groups 1, 2, and 3 in the course of and after treatments.

Testicular volume data are listed in Table 3. No significant difference emerged among or within groups before, during, or after therapy.

Cumulative percentage rates of naturally achieved pregnancies before, during, and after treatment are presented in Figure 1. At the end of the follow-up, group 2 had a significantly higher pregnancy rate compared with group 1 ($\chi^2 = 20.795$, $P < .01$), and group 3 had a significantly increased pregnancy rate compared with group 2 ($\chi^2 = 5.743$; $P < .05$).

Side effects were negligible and never resulted in therapy suspension. Two patients from group 1, 2 patients from group 2, and 3 patients from group 3 reported mild euphoria, and 2 patients from group 1 and 2 patients from

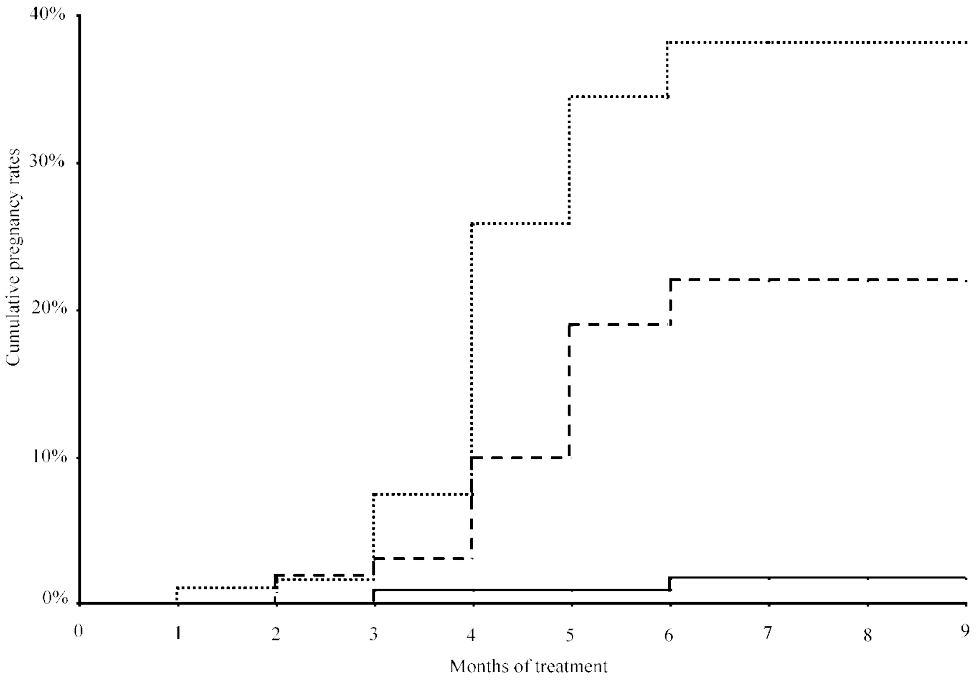

Figure 1. Cumulative percentage rates of naturally achieved pregnancies before, during, and after treatment. Solid line indicates group 1 (placebo) data; dashed line, group 2 (L-carnitine/acetyl-L-carnitine); dotted line, group 3 (L-carnitine/acetyl-L-carnitine + cinnoxicam) data.

group 2 reported mild epigastralgia and nausea ($\chi^2 < 1$; *P* not significant).

Discussion and Conclusions

These results allow us to maintain that L-carnitine/acetyl-L-carnitine + cinnoxicam proved significantly more effective than L-carnitine/acetyl-L-carnitine alone and than the placebo in increasing sperm concentration, motility, and morphology of idiopathic and of grades I, II, III, and IV varicocele-associated OAT males for as long as they were delivered. When the therapies were suspended, sperm patterns returned to baseline. Group 3 treatment significantly increased sperm patterns of grade IV varicoceles when compared with the placebo group, but to a significantly lesser extent when compared with group 3 lower grade varicoceles or to group 3 OATs. No drug proved active in grade V varicoceles. The higher the degree of varicocele is the lower the benefit form therapy. It may be postulated that a putative harmful agent could be inactivated by carnitines and cinnoxicam in low degree varicoceles while therapy is administered but not in high degree varicoceles.

The highest improvement in pregnancy rates occurred in group 3, and group 2 improved more than group 1. When drug administration was suspended, pregnancy rates did not increase any further in any group.

Only negligible side effects emerged, with no significant differences among the 3 groups. Patients (287/325) reported more abundant or easier defecation or both on the day in which suppositories were used, and they did not notice any appreciable discomfort; therefore, this occurrence was not reported as a side effect, and as a result, no significant difference emerged between cinnoxicam and glycerine. We used a suppository dosage much lower than the manufacturers' suggestions, which indicated a twice daily cinnoxicam dosage and a glycerine dosage not exceeding once per day (Federazione Nazionale Farmacisti, 2003).

Parametric tests have been preferred for the statistical analyses of sperm concentration, motility, and morphology, because nonparametric tests allocate the data in numbers of ranks that cause individual differences to go unnoticed and homogenize the treatment differences. A fundamental requirement of parametric tests is the equality of the standard deviations of the data series. Randomized block, paired data tests analyze the individual as a potential origin of variation. Significant differences were found in the course of active drugs and placebo administration. This means that there are individual responses to placebo and active drugs, corroborating on the one hand the knowledge that spontaneous variations in sperm patterns can occur (Lenzi et al, 2003) and, on the other hand, the hypothesis that idiopathic OAT might be a mosaic of infertilities (Bonanomi et al, 2002; Foresta et al, 2002). As a consequence, standard deviations of natural data of sperm concentration increased proportionally by their

mean, and logarithmic transformation of the data was indicated. Motility and morphology natural data are percentages; their standard deviations tend toward zero for the extreme values (0% or 100%) and increase for the central values (50%): extreme low values of standard deviation were found in untreated patients, and more central values were found in the course of drug treatments. This means that angular transformation is appropriate. The Kolgorov-Smirnov and the Shapiro-Wilk tests indicated a nonnormal distribution of the natural data, but when these tests were applied to transformed data, they indicated a Gaussian distribution (Snedecor and Cochran, 1987).

Carnitine dosage was chosen according to previous literature. Lenzi et al (2003) used L-carnitine 1 × 2 g/d; Vicari and Calogero (2001) used acetyl-L-carnitine (1 g/d) + L-carnitine (1 × 2 g/d). Previous preparatory tests indicated that acetyl-L-carnitine/L-carnitine proved significantly more effective than L-carnitine alone and as effective as Cinnoxicam alone. Initially, we considered using oral and injected NSAID, but their affect on sperm count has proved lower than suppositories. No specific research on delivery has been conducted, but a more direct effect of suppositories on seminal plasma might be presumed because of rectal-prostatic lymphatic pathways (Gattuccio et al, 2000). To date, 4 NSAIDs are available in Italy as suppositories (diclofenac, nimesulide, piroxicam, and cinnoxicam). In previous tests, cinnoxicam was significantly more active than the other 3 in increasing sperm count, probably because it is lipophylic, which facilitates absorption (Berti et al, 1992; Bertè and Richelmi, 1998).

The cinnoxicam dosage was chosen from several possibilities (2 suppositories per day to 1 per week), and 1 every 4 days was the dosage that increased sperm count the most. Confirming this kind of "spot therapy," an experimental model showed that chronic treatment with NSAIDs at low doses improved sperm quality and fertility (Loescher et al, 1988), whereas in vitro experiments showed that a high cinnoxicam concentration inhibits sperm motility (Mangano et al, 2000).

Increased reactive oxygen species (ROS), subsequent lysosomal permeabilization, and apoptosis have been implicated as the final common pathway of most OAT cases. Increased ROS were found in tubular and seminal plasma of idiopathic OAT, OAT associated with varicocele, and male accessory gland inflammation. Increased ROS provoked membrane lipoperoxidation, leading to motility and morphology alterations and even to cell death (Sale and Agarwal, 2002). Spermatids and mature spermatozoa are deemed highly sensitive to ROS because their membranes are particularly rich in polyunsaturated lipids. Superoxide dismutase, catalase, glutathione-dependent peroxidase activity, reduced glutathione (GSH), tocopherol, and ascorbic acid concentrations were the highest of all the human fluids found in tubular and seminal plasma. A preventive measure against high ROS has been advised for tubuloseminal plasma for male gametes (Sale and Agarwal, 2002). A return to physiological ROS concentrations in the course of carnitine administration is associated with an improvement in sperm patterns in varicoceles (Gattuccio et al, 2000), idiopathic OATs (Lenzi et al, 2003), and postinflammatory OATs (Vicari and Calogero, 2001). Therefore, carnitines have been reputed as anti-ROS drugs (Gattuccio et al, 2000; Vicari and Calogero, 2001; Lenzi et al, 2003).

In humans and experimental models, carnitines have several activities that might be useful for the male gamete. Free radical production is reduced by depleting fatty acid peroxidation; carnitines restore the phospholipid composition of mitochondrial membranes; carnitines enhance cellular energetics in mitochondria, increasing cytoplasmic acetyl-coenzyme A concentration through the higher availability of acetyl groups, resulting in an increase of mitochondrial respiration and monoamine-oxidase activity and thus increasing the metabolism of histamine; and carnitines stabilize cell membrane fluidity by regulating phospholipid levels and reducing ceramide production and insulinlike growth factor 1. Carnitines prevent cellular death and apoptosis. Carnitines display most of their activity in the tubular microenvironment, rather than in epididymal functions (Gattuccio et al, 2000; Vicari and Calogero, 2001; Lenzi et al, 2003).

Carnitine administration increases E2 prostaglandin concentration (Vicari and Calogero, 2001), which affects sperm count (Ito et al, 1982; Fuse et al, 1984; Bendvold et al, 1987). Mast cell count increased in bioptic testicle specimens from idiopathic infertile males (Apa et al, 2002), and long-term administration of tranilast (a mast-cell blocker) enhanced semen parameters in OAT patients (Hibi et al, 2002). NSAIDs might improve sperm count: polyzoospermia and OAT are associated with lowered and increased prostaglandin content of tubuloseminal plasma, respectively. In addition, NSAIDs stabilize lysosomal membranes, thereby partially preventing apoptosis (Ito et al, 1982; Fuse et al, 1984; Bendvold et al, 1987). These findings could explain cinnoxicam's complementary mechanism when it is administered in association with carnitines.

Conventional anti-ROS drugs (vitamin E, ascorbic acid, GSH, and essential fatty acids) failed to enhance sperm patterns in prospective controlled trials (Rolf et al, 1999; Comhaire et al, 2000; Bolle et al, 2002; Wong et al, 2002). Carnitines display a number of activities, and they restore the physiological concentration of ROS by acting on the Krebs cycle (Gattuccio et al, 2000; Vicari and Calogero, 2001; Lenzi et al, 2003). Conventional antioxidant drugs interact directly with the substrate (ROS) and decrease ROS regardless of their concentration (Com-

haire et al, 2000; Bolle et al, 2002; Rolf et al, 2002). Whether this peculiar activity on ROS concentration is the only explanation of the different activities of carnitines and conventional anti-ROS drugs is only speculative. Either way, an increased ROS concentration is detrimental for male gametes, but a low ROS concentration down-regulates the capacitation and acrosomal reaction of male gametes (Bart Fauser, 2003).

In this study, pregnancy rates per couple month were 0.3% in group 1, 3.6% in group 2, and 6.3% in group 3. Group 1 and group 2 pregnancy rates per couple month fit with previous reports. The spontaneous pregnancy rate per couple month of placebo-treated patients in the prospective trials for idiopathic OAT ranges from 0% to 1.4% in the presence of an apparently fertile female partner (WHO, 1989, 1992; Rege et al, 1997; Kamischke et al, 1998; Rolf et al, 1999; Adamapoulos et al, 2003; Lenzi et al, 2003; Zawackzi et al, 2003). Pregnancy rate in couples waiting for assisted reproduction techniques (ART) because of male OAT was 0.13% per month (Matorras et al, 1996). Mean couple infertility duration ranged from 14 to 18 months in these studies. These rates were lower than the overall pregnancy rates of the couples waiting for ART (Bart Fauser, 2003). Male OAT decreases the monthly chance of conception in a dose-dependent manner, adding its effect to the duration of infertility (Jansen, 1993). As confirmation, an increase of sperm concentrations in OAT-infertile males has been associated with disproportionately higher fecundity (Adamapoulos et al, 2003). The studies of carnitines and male OAT used acetyl-L-carnitine or L-carnitine separately: only Vicari and Calogero (2001) used the association L-carnitine/actetyl-L-carnitine and obtained a 3.9% pregnancy rate per couple month.

Despite the size and duration of this trial, the population of patients with idiopathic dyspermia or with dyspermia associated with low-grade varicoceles is much larger: patients with sperm concentration lower than 5 000 000/mL or patients with isolated defects of motility or of morphology who have normal concentration have been excluded form this study because pretrial tests indicated that our therapy proved ineffective in them. Consequently, the results of this study cannot be extrapolated to all patients seen in general practice for dyspermia and infertility. A survey of the data of Adamapoulos et al (2003) and Wong et al (2002) indicated that their trials predominately treated patients with isolated alterations of motility, morphology, or both who have normal concentrations of sperm. Therefore, it might be interesting to look for a comparison of or an association between the different therapies.

This paper has addressed subclinical varicoceles, whose therapy is controversial (Jarow, 2001) and which cannot be extrapolated to the whole population of OAT associated with varicoceles. Even though some authors still do not consider varicocele surgical repair a treatment for male infertility (Evers and Collins, 2003), there is agreement about the favorable effects of large varicocele repair, which persists over time and could be easily performed as outpatient surgery (Gattuccio et al, 2000; Jarow, 2001; Cavallini et al, 2003). With the continued presence of varicoceles, there is ongoing damage of seminiferous tubules, which can be repaired by surgery (Greenfield et al, 2002). It is unknown whether it can be influenced by medical therapy. Atrophic testicular volume has been shown to increase after surgery for high-grade varicoceles (Gattuccio et al, 2000), and it has been regarded as a proof of increased spermatogenesis. The volume of atrophic testicles with grade IV and V varicoceles did not change significantly in the course of drug administration, even though in grade IV/group 3 varicoceles with atrophic testicles the sperm count increased, but to a lower extent than in lower varicocele grades. The average cost for each naturally induced pregnancy in varicocele patients was €2133.60 and €1086.40 in idiopathic OATs. An overview of our data indicated that the difference is due to the poor activity of the drugs in high-grade (grades IV and V) varicoceles.

Sixty-four group 3 patients reached the WHO normal range of sperm analysis in the course of therapy, but only 41 conceived naturally. Because semen quality is positively related to ART outcomes (Bart Fauser, 2003), it should be interesting to assess the role of L-carnitine/acetyl-L-carnitine + cinnoxicam in those couples in which semen quality improved but who did not conceive naturally and ARTs were performed.

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