### ORIGINAL ARTICLE

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# Dietary supplementation with a novel L-carnitine multi-micronutrient in idiopathic male subfertility involving oligo-, astheno-, teratozoospermia: A randomized clinical study

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### Abstract

**Objective:** To study the influence of a multi-component nutrient dietary supplement on sperm parameters and pregnancy rates in idiopathic male infertility (IMI) with oligo-, astheno-, and teratozoospermia.

**Design:** A randomized, double-blind, placebo-controlled, prospective, parallel arms (1:1 allocation ratio), multi-center clinical trial.

Settings: Eight urology/reproductive health clinical centers located in Ukraine.

**Patients:** Eighty-three males aged 21-50 years with IMI and at least 1 of 3 abnormal values: total sperm concentration < 15 million/ml or/and spermatozoa progressive motility < 32% or/and forms with normal morphology < 4%.

**Intervention(s):** Patients were randomly allocated verum test dietary supplement (TDS) containing L-carnitine/acetyl-L-carnitine, L-arginine, glutathione, co-enzyme Q10, zinc, vitamin B<sub>9</sub>, vitamin B<sub>12</sub>, selenium, or placebo 1 time daily for 6 months.

**Main Outcome(s):** The primary outcome measure was the percentage of normal spermiograms (concentration  $\geq$  15 million/ml and  $\geq$  32% of spermatozoa with progressive motility and  $\geq$  4% of normal forms) at month 0, 2, and 4. The percentage of pregnancies served the secondary outcome endpoint. Differences between the groups were assessed in z-test for proportions.

**Results:** All males finished the study. At month 4, 29/42 (69.0%) males in the verum and 9/41 (22.0%) had normal spermiograms (P < .001). The percentage of spontaneous pregnancies in the verum group was greater than in the placebo group (10/42, 23.8% vs. 2/41, 4.9%, respectively, P = .017). There were no reportable supplementassociated adverse events.

**Conclusion:** Specific multi-nutrient combination L-carnitine/L-acetyl-carnitine, L-arginine, glutathione, co-enzyme-Q, zinc, folic acid, cyanocobalamin, and selenium can improve sperm quality in males with IMI and increase pregnancy rates.

### KEYWORDS

antioxidants, dietary supplement, idiopathic, male infertility

Clinical trial registration number: NCT03588949.

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### 1 | INTRODUCTION

It is believed that about half of the childless couples who suffer from infertility do not conceive due to a male factor.<sup>1</sup> In 44% of men, there are no identifiable medical conditions that could explain infertility and these cases are classified as idiopathic male infertility (IMI).<sup>2</sup> Because of the "idiopathic" nature of the condition, there are no evidence-based treatments for IMI, and most therapeutic efforts rest on an empirical approach.<sup>3</sup>

Several studies have shown variable benefits of nutrients in the management of IMI and improvement of sperm tests. L-carnitine (LC) and L-acetyl-carnitine (LAC) are two forms of a substance that specifically accumulates in the epididymal lumen in the diet of males with IMI and abnormal sperm values. Inclusion of them has led to an increase in sperm concentration as well as total and progressive sperm motility and normalization of cell morphology with the effect being more pronounced in males with lower baseline spermiogram values.<sup>4-7</sup> One study has demonstrated stimulatory effect of LC on progressive sperm motility in prostato-vesiculo-epididymitis and elevated seminal leukocytes after pre-treatment with non-steroidal anti-inflammatory drugs.<sup>8</sup> Trying to replicate these results, a study in which a limited number of patients with idiopathic asthenospermia were enrolled failed to demonstrate the effect of L-carnitine on sperm motility and total motile sperm counts.9 A meta-analysis of three selected trials, comparing carnitine therapy with placebo treatment, concluded there might be a significant improvement in total and progressive sperm motility as well as sperm cell morphology without significant changes in sperm concentration.<sup>10</sup>

Comparing with a group of patients taking L-carnitine alone and men taking L-carnitine with other nutrients (enzyme cofactors, vitamins, trace minerals) showed a positive effect on sperm concentration, progressive motility, and normal morphology of spermatozoa.<sup>11</sup> A recent randomized trial has documented that a multiple combination of nutrients improves progressive motility and vitality of spermatozoa in IMI and idiopathic oligoasthenoteratozoospermia.<sup>12</sup> These studies have not reported pregnancy as an outcome, while the updated Cochrane systemic review of evidence shows that antioxidants taken by men may lead to increased clinical pregnancy rates.<sup>13</sup>

In the present study, we aimed to assess whether multi-component nutrient dietary supplement can influence both sperm parameters and pregnancy rates in IMI with oligo-, astheno-, and teratozoospermia.

### 2 | MATERIAL AND METHODS

### 2.1 | Study design

A randomized, double-blind, placebo-controlled, prospective, parallel arms (1:1 allocation ratio) study was conducted at 8 centers in Ukraine with urology or reproductive health departments (Medical Center "Astar," Lviv; Precarpathian Center of Reproductive Health, Ivano-Frankivsk; Mykolaiv State Clinical Hospital, Mykolaiv; Lviv State Center for the Reproductive Health, Ukraine; Private Urologic Practice, Mykolaiv; Medical Center "ExtraMed," Ivano-Frankivsk; Lviv State Hospital, Lviv). The protocol informed consent form and other materials of the study were approved by the local Ethical Committees at each center.

The study was conducted in accordance with the principles of the Declaration of Helsinki, and the privacy rights of patients were respected in compliance with Good Clinical Practices throughout the study. Before the study enrollment, patients were familiarized with the study procedures and consider their participation. A signed informed consent was obtained from all patients. This prospective study was registered at ClinicalTrials.gov on July 17, 2018, before the first patient signed the informed consent form (identifier: NCT03588949).

Time points of the study included the screening period, baseline, and final doctor visits, three laboratory visits, three text remainders send via mobile short message service (SMS), and a standard phone call (Figure 1).

During the screening period, eligibility of patients was assessed and medical records were evaluated for exclusion criteria. On the baseline visit, demographic data were collected, and complete urologic examination was performed. Seven through 0 day before the baseline visit, and at month 2 and month 4, sperm analyses were performed. During baseline visit, patients received the Test Dietary Supplement (TDS), chart of use of the TDS (CTDS), and were advised to start taking the TDS on the day or the next day after baseline visit. SMS messages provided reminders about forthcoming laboratory or doctor visits. During the final doctor visit, the remaining TDS was returned and counted for an assessment of compliance. The 6-month intervention and follow-up period was finished with a standard phone call interviewing compliance with TDS intake, adverse events, screening tests for conception, and ultrasound confirmation of pregnancy. When pregnancy occurred, the investigator requested pertinent confirmation from the patient's gynecologist/obstetrician.

### 2.2 | Study population

Couples who had complete clinical and laboratory evaluation for infertility were selected from the patient databases. Male information included endocrine tests (luteinizing hormone, LH; follicle stimulating hormone, FSH), infections (HIV, gonorrhea, syphilis, results of polymerase chain reaction/culture of spermatozoa/discharge), ultrasound of genitals (testes, seminal vesicles, prostate), and previous treatment of infertility (gonadotropin-based drugs, gonadotropinreleasing hormone drugs, antiestrogens, aromatase inhibitors, dietary supplements). Endocrine tests, as well as other laboratory evaluations, were considered valid if they were performed for the last 12 months before enrollment of patients in the study. Female history reviewed number of pregnancies, abortions, documented or suspected amenorrhea/oligomenorrhea, cervical stenosis, mucous pathology, uterine polyps or fibrosis, congenital anomalies of uterus, vagina, fallopian tube pathology, pelvic inflammatory disease,



FIGURE 1 Design and chronology of the study. TDS, test dietary supplement; SMS, short message service communication

endometriosis, pelvic ultrasound (ovaries, tubes, uterus), previous treatment of infertility, and infection tests.

The inclusion criteria were informed consent form signed, age 21-50 years, idiopathic male infertility defined as absence of conception in a couple having a regular unprotected intercourse for 12 months<sup>14</sup> with a woman without evident pathology that could cause infertility, oligo- (sperm concentration < 15 million/ml) and/ or astheno- (<32% forms with progressive motility) and/or terato-zoospermia (<4% of sperm cells with normal morphology), affirmed availability throughout the study period and a mobile phone.

The exclusion criteria were allergy to any component of the TDS, known genetic, anatomical, endocrine, and inflammatory or traumatic testicular cause of male infertility; known or suspected genetic, anatomical, endocrine, and inflammatory cause of female infertility; inflammatory bowel disease; moderate-to-severe disease of any systems; sexually transmitted diseases; alcohol or drug addiction of any couple counterpart as suspected by investigator; difficulty understanding the study requirements as judged by an investigator; use of any investigational product within the previous 3 months before entering the study; and use of any drugs that stimulate or suppress spermatogenesis within previous 3 months.

### 2.3 | Intervention and blinding

Random numbers were generated online with no restrictions to randomization by the statistician using the web site Randomization. com.<sup>15</sup> These numbers were printed and steaked on the verum or placebo boxes with TDS. Afterward, the statistician sent labeled boxes to the centers. The investigators distributed TDS to the patients by sequential selection of the boxes with random numbers.

The investigators and patients were concealed, which type of the TDS, verum or placebo, was selected.

One dose (sachet) of the verum TDS contained 12 Kcal, L-carnitine/L-acetyl-carnitine—1990 mg, L-arginine—250 mg, glutathione—100 mg, co-enzyme Q10—40 mg, zinc—7,5 mg (75% of recommended daily allowance, RDA), vitamin  $B_9$ —234 mcg (117% RDA), vitamin  $B_{12}$ —2 mcg (80% RDA), selenium—50 mcg (91% RDA), excipients sorbitol, maltodextrin, orange/beta-carotene colorant, saccharide, acesulfame potassium, and silicium dioxide. The placebo contained the excipients orange/beta-carotene colorant, citric acid anhydride, sorbitol, silicium dioxide, magnesium stearate, and maltodextrin. Both placebo and verum boxes with sachets of the TDS looked the same, and their content was similar on smell, texture, and color.

One dose per day of the TDS was taken for 6 months (180 doses total) in the evening 30 minutes before the last planned meal. If pregnancy developed before month 2 or 4 laboratory visits, male was requested to continue TDS intake until all sperm analyses were available. The fact of the intake was recorded by the participant in the CTDS. Compliance was reassessed during scheduled phone calls, and remaining number of sachets was counted after collection of boxes at the end of the 6-month observation period. The number of the remaining TDS was compared with the number of doses recorded in the CTDS. A man was considered compliant if he consumed more than 90% of the planned number of doses.

To avoid biases with study outcomes, any drugs or dietary supplements with known or suspected action on male spermatozoa (gonadotropin-based drugs, gonadotropin-releasing hormone, antiestrogens, aromatase inhibitors, herbal, micronutrient, vitamins, fish oil) or female fertilization potential were excluded. During baseline visit, patients learned about drugs with known negative influence on spermatogenesis (testosterone, 5-alpha-reductase inhibitors,

alpha-blockers, PDE5 inhibitors, selective serotonin reuptake inhibitors, ketoconazole).

Any restrictions to the normal diet were not advised. Study participants remained on their diet, which they practiced before the study. After enrollment in the study, sex practices were not restricted or modified with exception of withholding from sexual activity for 3-5 days before the sperm analysis to be performed.

#### 2.4 Semen analysis

The participants were supplied with a leaflet reminding timelines of sperm analysis, and listing factors that could interfere with sperm evaluation.

After 3-5 days of abstinence, semen was collected at the participating laboratory and was available for the analysis within 5-10 minutes. After liquefaction, sperm concentration, progressive motility, and normal sperm forms were assessed according to WHO 5 ed. (2010) recommendations.<sup>16</sup> For examination of sperm counts, an improved Neubauer hematocytometer was used. Motility of spermatozoa was evaluated by direct microscopic examination at magnification of the microscope ×400. Papanicolaou staining technique was used for description of sperm morphology by examining the slide with brightfield optics at ×1000 magnification with oil immersion, using schematic drawings of some abnormal forms of human spermatozoa in the manual.<sup>17</sup> Results of the analyses were printed out on the center template in local language and transferred on the standard English language form.

### 2.5 | Adverse events

Although the investigational TDS does not qualify as a medicinal product, screening for intolerance events was performed for all participants. During visits and phone calls, the investigator asked openended questions about food intolerance events and listed their most probable manifestations (abdominal distension, abdominal pain, bloating, constipation, diarrhea, nausea, pruritus, rash, and urticaria). The degree of association of the event with the TDS was graded as probable, possible, remote, and unrelated.

### 2.6 | Outcome measures

The primary outcome measure was the rate of normalization of spermiogram at the 2nd and 4th month. Normalization of the spermiogram was defined as sperm concentration  $\geq$  15 million/ml and  $\geq$  32% of spermatozoa with progressive motility and  $\geq$  4% of normal forms<sup>-</sup>

The secondary outcomes were sperm concentration (million/ml), progressive motility (%), and normal sperm morphology (%) at baseline, month 2 and month 4; absolute change from baseline to 2nd and 4th months in concentration, progressive motility, and normal sperm morphology; and number and percent of any of 7 abnormal spermiogram (volume < 1.5 ml, concentration <  $15 \times 10^6$ /ml, total sperm number  $< 39 \times 10^6$ /ejaculate, total motility < 40%, progressive motility < 32%, normal forms < 4%, viability < 58%) at 0, 2, and 4 month.

Pregnancy rates (%) and time to conception (days) served as tertiary outcomes. Pregnancy was diagnosed with ultrasound, and time to conception was defined as the time from the first day of intake of the TDS to the first day of the last menstruation. There was no change to trial outcomes measures.

#### Sample size and statistical analysis 2.7

It was hypothesized that after 4 months of intake of the TDS, the verum group would experience normalization of spermiogram in 55% of cases, while in the placebo group this normalization would occur in 25%. Assuming 1-beta error 0.80 and type I error alpha 5%, 80 subjects had to be enrolled to achieve sufficient power. There were no statistical assumptions for other outcome measures.

Absolute number and percent were used to describe race, education, employment, alcohol consumption, smoking habit, and food intolerance events. Mean and standard deviation described age, body mass index, time from diagnosis of infertility, time to conception (last day of the previous menstruation), and spermiogram values. Normality of data distribution was assessed with Kolmogorov-Smirnov test. Difference between the groups in percent data was assessed in z-test, and between means in two-tailed t test. To control for confounders, ANCOVA analysis was used with baseline values for sperm concentration, progressive motility, and normal morphology as covariates. All tests were performed with software Statistica 9 (StatSoft., Inc, OK).

There were no changes made to methods and interim analyses after the trial commencement.

#### RESULTS 3

Screening and enrollment of males occurred continuously from September 2018 to April 2019 (Figure 2).

Of 210 initially screened males, 33 met exclusion criteria, and 94 declined to participate with 53 patients expressing unclear promise to finish whole observation period. Eighty-three males were randomized and allocated verum (n = 42) or placebo supplementation (n = 41). Due to careful selection of well-motivated patients, no males left the 6-month observation period of the study.

At baseline, the verum and placebo groups were comparable on demographic and medical characteristics (Table 1).

Most men were aged 30-40 years with time from the diagnosis of infertility ranging 4-6 months. Women were 3-4 years younger than males. Body mass index varied around 24-25 kg/m<sup>2</sup> and was normal in all males. Around half of the participants had a university education, and most reported low alcohol consumption. In both the verum and placebo groups, approximately one-third were smokers.

## **FIGURE 2** Participants flow diagram. TDS, test dietary supplement





	Verum group	Placebo group
Characteristic	n = 42	n = 41
Age, years, mean (SD)	32.5 (6.1)	32.7 (5.2)
Time from diagnosis, months, mean (SD) <sup>a</sup>	6.1 (8.7)	4.4 (6.8)
BMI, kg/m <sup>2</sup> , mean (SD)	25.3 (4.4)	24.6 (2.6)
Age of woman, years, mean (SD)	29.0 (4.6)	29.2 (4.1)
Education		
School, n (%)	17 (40.5)	16 (39.0)
University, n (%)	20 (47.6)	22 (53.7)
Other, n (%)	5 (11.9)	3 (7.3)
Employment		
Full-time, n (%)	28 (66.7)	31 (75.6)
Part-time, n (%)	8 (19.0)	7 (17.1)
Unemployed, n (%)	6 (14.3)	3 (7.3)
Alcohol consumption		
No consumption, n (%)	1 (2.4)	0 (0.0)
Low, n (%)	26 (61.9)	27 (65.9)
Moderate, n (%)	15 (35.7)	12 (29.3)
High, n (%)	0 (0.0)	2 (4.9)
Smokers, n (%)	16 (39.0)	14 (34.1)

<sup>a</sup>Time lapsed from the diagnosis of infertility.

Before entering the study, none of the males received gonadotropin-based drugs, gonadotropin-releasing hormone, antiestrogens, or aromatase inhibitors. However, 18.1% of patients (15/83) (9/42, 21.4% in the verum and 6/41, 14.6% in the placebo group) received dietary supplements for infertility before the study. Six patients received one dietary supplement (4/42, 9.5% in the verum and 2/41, 4.9% in the placebo group), 8 males—two dietary supplements (5/42, 11.9% in the verum and 3/41, 7.3% in the placebo group), and 1 (placebo)—three dietary supplements. Mean total duration of intake of dietary supplements was 95.2 (41.4) days in the verum and 88.8 (45.5) in the placebo group. Consumption of all dietary supplements was stopped at least 3 months before the study enrollment. No drugs or supplements were received during the study.

According the count performed on the remaining sachets at the final visit and assessment of the CTDS, all participating males were compliant with TDS intake.

### 3.1 | Evaluation of efficacy

At entry, no males had all three main spermiogram values (sperm concentration, progressive motility, normal sperm morphology) normal. At month 2, 26/42 (61.9%) males in the verum and 8/41 (19.5%) males in the placebo group had all 3 variables normal (P < .01). At month 4, in the verum group patient with normal spermiograms outnumbered those in the placebo (29/42, 69.0% vs. 9/41, 22.0%, respectively, P < .001). At month 4, the difference in normalization of spermiogram was 47.0%.

At month 2 and 4, all evaluated spermiogram values were better in the verum group, except for the month 2, when difference in normal morphology was not significant (Table 2).

ANCOVA analysis confirmed that after controlling for confounders (baseline values of sperm concentration, progressive motility, normal morphology), the difference between the verum and placebo groups remained significant for corresponding values obtained at month 2 (concentration, P < .001; progressive motility, P < .001;) and 4 (concentration, P < .001; progressive motility, P < .001; normal morphology, P < .012).

Absolute change from baseline in mean values was significant for all main parameters evaluated at any time point, except for sperm morphology that was statistically different from between the groups only at month 4 (Figure 3).

The percentage of patients with 0, 1, 2, and 3 or more abnormal spermiogram values positively evolved over the study period in the verum group (Figure 4).

At baseline, no males had normal spermiogram values for all parameters, and spermiograms with 3 or more abnormal values were seen in 40.5% the verum group, and 46.3% in the placebo group. At month 2, the number of patients with 3 or more abnormal values reduced significantly in the verum group (16.7%), while in the placebo group, the relevant number increased up to 53.7% (P < .05). At month 4, the number of patients in the verum group with 3 or more abnormal spermiogram values reduced more than twofold, as compared with month 2, whereas in the placebo group it did not change significantly (P > .05). The difference in proportions between the groups at month 4 was significant (P < .001). The number of patients with all normal spermiograms showed reciprocal trend. At month 2, in the verum group 47.6% of spermiograms became normal vs. 17.1% in the placebo group (P < .05). At month 4, the number of normal spermiograms was 64.3% in the verum and 19.5% at placebo group (P < .001). During observation period of 6 months, couples in the verum group got pregnant in greater proportion (10/42, 23.8% vs. 2/41, 4.9%, P < .05). All pregnancies were achieved spontaneously, except for 1 pregnancy in the verum group, which was induced by the in vitro fertilization technique.

There were no reportable product-related intolerance events during the intake of the TDS.

### 4 | DISCUSSION

In our study, we used the percentage of spermiograms with normal sperm concentration, and progressive motility, and normal morphology the primary outcome measures. This triplet was chosen because abnormal values of its components constitute a core for the definition of oligo-, astheno-, and teratozoospermia accompanying idiopathic male infertility.<sup>3</sup> It describes different and relatively independent aspects of sperm quality, and may serve as surrogate measures of infertility in men, as every component has been found in significant positive correlation with in vitro fertilizing activity of spermatozoa.<sup>17</sup> These parameters have been most frequently reported in trials of antioxidants in male infertility with sperm abnormalities.<sup>14</sup>

In the verum group, the percentage of normalized spermiograms was significantly greater after exposure to the investigational TDS. This was corroborated by the significant change of all three parameters at month 4 as compared with baseline values. Not being reported simultaneously as a combined endpoint, sperm concentration, progressive motility, and normal morphology were extensively studied after use of LC/LAC and other nutrients.

Most trials exploring LC/LAC reported its stimulatory action on progressive sperm motility.<sup>4-8</sup> Meta-analysis of 3 placebo-controlled studies involving 199 patients revealed highly significant mean difference between the exposure and control groups.<sup>14</sup> This analysis did not encompass discouraging pilot results obtained in a group of 21 males (12 verum vs. 9 placebo) with 10%-50% of progressively moving spermatozoa taking LC/LAC for 24 weeks.<sup>9</sup> In the literature, improvement in progressive sperm motility remained one of the most frequently reported positive effects of LC/LAC, whether in idiopathic sperm abnormalities or in inflammatory illnesses.<sup>18</sup> In prostato-vesiculo-epididymitis, characterized by the high concentration of reactive oxygen particles, LC/LAC were administered after conventional antibacterial and anti-inflammatory treatments with

TABLE 2 Absolute baseline, months 2 and 4 follow-up sperm concentration, progressive motility, and normal morphology

Characteristic	Verum group n = 42	Placebo group n = 41	Verum group n = 42	Placebo group n = 41	Verum group n = 42	Placebo group n = 41
Concentration, 10 <sup>6</sup> /ml, mean (SD)	40.8 (29.7)	41.4 (30.6)	62.2 (33.6)	43.8 (23.0)**	78.7 (39.0)	44.0 (26.7)***
Progressive motility, %, mean (SD)	21.2 (7.1)	21.8 (8.6)	34.1 (11.5)	24.0 (10.3)***	40.8 (16.8)	26.1 (15.8)***
Normal morphology, %, mean (SD)	12.6 (12.8)	12.2 (8.2)	14.1 (9.4)	12.6 (7.7)	21.1 (16.0)	14.1 (11.2)*
Normal morphology, %, mean (SD)	12.6 (12.8)	12.2 (8.2)	14.1 (9.4)	12.6 (7.7)	21.1 (16.0)	14.1 (11.2)*

\* $P \le .05$ ; \*\* $P \le .01$ ; \*\*\* $P \le .001$  as compared with verum group at a time point.

FIGURE 3 Change in sperm concentration, progressive motility, and morphology 2 and 4 months after starting the test dietary supplement intake. Data present as mean (middle point), confidence interval (box), and standard deviation (whiskers). Verum group boxes present in brown, placebo—in green color. A footnote table shows absolute change from the baseline through months 2 and 4 in sperm concentration, progressive motility, and morphology ANDROLOGY 
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a beneficial effect of sperm motility and viability.<sup>19</sup> A summary of 13 trials with placebo controls or no treatment, embracing 884, still yielded highly significant effect.<sup>14</sup> Available data suggest that carnitines can play a leading role in improvement of sperm motility via acceleration of  $\beta$ -oxidation of long-chain fatty acids in mitochondria and production of energy necessary for locomotion.<sup>19</sup>

No stimulation on the progressive sperm motility was found in the small sample size studies with co-enzyme Q10, folic acid, zinc, or combination of zinc and folic acid.<sup>20-22</sup> In a recent large-scale randomized multi-center trial exploring the role of 6-month folic acid (5 mg/daily) and zinc (30 mg/daily) supplementation in infertility couples, authors demonstrated no effects on any standard spermiogram parameters, including progressive sperm motility.<sup>23</sup> The doses of folic acid and zinc varied around recommended daily allowance and were times lesser in our study than in the referenced one. Therefore, we neither could extrapolate documented spermiogram changes to these dietary components nor exclude the likelihood of spermiogram modifications by interplay of zinc and folate with other components of the formula used or importance of their lower doses. There is a growing concern high doses or overuse of antioxidants

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and possible inflicted harm.<sup>24</sup> It is postulated that excessive intake of antioxidants can shift tissues into a more reduced state called "reductive stress" or even increase concentration of reactive oxygen species, an "antioxidant paradox." The consequence of this may be infertility associated with DNA damage, reduction in sperm quality and function, absence of spontaneous pregnancy, which was explicitly described in one infertile couple, antioxidant abusers, who got pregnant after cessation of antioxidants.<sup>25</sup>

Selenium and L-arginine taken separately for 3 months by males with oligo- or asthenospermia promoted progressive movement of spermatozoa in 2 other clinical trials.<sup>25,26</sup> Glutathione, an important compound in sperm antioxidant defense system, also demonstrated a positive role on sperm motility.<sup>27-29</sup> Together with selenium, it is essential for production of glutathione peroxidase, a structural protein comprising the half of the mitochondrial capsule in the mid-piece of mature spermatozoa. Lack of glutathione/selenium can lead to instability of the mid-piece, resulting in defective motility.<sup>30,31</sup>

The listed components being used together may theoretically enhance function of carnitines. In the prospective open-label study comparing carnitines in combination with L-arginine, zinc, tocopherol, glutathione, selenium, and co-enzyme Q10 vs. carnitines alone showed two times greater effect.<sup>12</sup> This supports the hypothesis that the increase in sperm motility induced by carnitines can be enhanced by the adjuvant nutrients, which may fail to produce a positive effect when administered separately.

Some of the components in the TDS may have improved sperm morphology. Zinc sulfate in a dose of 66 mg taken together with folic acid 5 mg a day for 26 weeks by the subfertile patients after varicocelectomy has been shown to significantly increase the percentage of spermatozoa with normal morphology at months 3 and 6.23 Zinc sulfate administered as solution 5 mg × 3 times daily (15 mg) for 3 months has been shown to very slightly increase the percentage of sperm cells with normal morphology,<sup>32</sup> but much higher daily dose of zinc (220 mg for 4 months) with 5 mg folate showed no benefits.<sup>33</sup> A systematic review and meta-analysis of the trials assessing a role of zinc and folic acid concluded about improvement of sperm morphology.<sup>34</sup> The content of zinc salt and folic acid in our TDS was lower than in the referenced studies, which prevent us from directly comparing our results to the data obtained by other researchers. Notably, the normalization of sperm morphology in the present study occurred at month 4 but not at month 2. This could be partially explained if a lower dose requires a longer time to eliciting the positive effect in de novo formed cells. Other constituent of the TDS, co-enzyme Q10 taken orally in a dose of 200 mg for 26 weeks may have increased normal sperm forms assessed with strict morphology criteria and total dose correlated positively with size of the effect. During 12-week washout period, normal sperm counts returned to the baseline levels.<sup>35</sup> The effect of normalization of sperm morphology cannot be ascribed to LC given as single substance, as its administration in a dose of 2 g for 13 weeks failed to improve sperm morphology in previous work.<sup>36</sup>

Our findings are in line with the results of previous studies where the effect of the antioxidants on sperm concentration was

described. In an earlier non-controlled multi-center study, a significant increase in sperm cells output was observed after intake of 3 g of LC for 4 months in patients with idiopathic asthenozoospermia.<sup>37</sup> Further controlled trials were inconsistently conclusive with a systematic meta-analysis of 247 cases suggesting that carnitines improve sperm concentration.<sup>14</sup> Co-enzyme Q10, folic acid, and selenium did not affect sperm density being administered as a single formulation,<sup>21-23,28</sup> while zinc with or without folic exerted a positive action on this parameter.<sup>23,34</sup> Multi-component antioxidant formulation, consisting of the listed components and carnitines, increased sperm concentration by 158% as compared with 64% increment, when carnitines were taken alone.<sup>12</sup> Significant changes in sperm concentration were achieved with the use of another multi-nutrient formula.<sup>13</sup> In the study reporting a global time-dependent decrease of sperm count, it was postulated that environmental oxidative insults can decrease sperm cell via apoptosis/direct degenerative action and result in decreased sperm concentration.<sup>38</sup> Conversely, an antioxidant dietary supplementation can plausibly alleviate oxidative influence and recover the decreased sperm counts.

The beneficial change in routine sperm parameters appears to have resulted in a statistically higher proportion of spontaneous pregnancies in the verum group as sperm concentration. Indeed, progressive motility and morphology are among primary predictors of male fertility.<sup>39</sup> Better pregnancy outcomes were reported for the prescribed separately zinc-, but not for folate- or co-enzyme Q10based supplements.<sup>7,23,40</sup> The cumulative data from 8 randomized clinical trials showed that carnitines increase proportion of pregnancies (OR = 4.10, P < .001).<sup>41</sup> Combination of zinc, folic acid, selenium, and other antioxidants given for 3 months helped to improve viable pregnancy rates as a result of 38.5% (verum group) vs. 16% (placebo group) successful embryo transfers after in vitro fertilization-intracytoplasmic sperm injection.<sup>42</sup> A double-blind, placebo-controlled trial investigated the effect of 6 months' supplementation with LC, LAC, and other micronutrients (zinc, co-enzyme Q10, selenium, folic acid, cyanocobalamin, ascorbic acid, plus fumarate, fructose, and citric acid) on pregnancy rates. Twelve pregnancies occurred during the follow-up period, 10/52 (19.2%) were reported in the supplementation and 2/52 (3.8%) in the placebo group.<sup>43</sup> Recent systematic review of evidence favored antioxidants as compared with placebo or no treatment in achieving pregnancies in subfertile males.<sup>14</sup>

The interplay of the specific components of the available multi-component formula is not yet clear and a matter of a multiarm step-by-step controlled clinical and laboratory evaluation. Indeed, seemingly co-orchestrating components may have overlapping, summarizing, mutually potentiating, or independent effects. Current analysis of these effects seems not pertinent for multi-nutrient formulae, as most results were obtained either for high-dose individual component that present in a lower quantity in combined formulations, or in different study designs. However, consistently better effects of the combined antioxidant formulations need further scientific investigation, whether they are due to interplay of constituents or just have a wider band to cover specific nutritional requirements in different patients. The strength of our study was its randomized, double-blind, placebo-controlled, prospective, parallel group, multi-center design, and pre-defined sample size. A major study limitation was absence of an assessment of the baseline and ongoing diet of males, including vegan preferences, which might theoretically result in a carnitine deficient condition. Indeed, dietary habits of males can significantly affect sperm quality and thereby fertilization potential.<sup>44</sup> Despite a nutritional nature of the research, recent review showed that of 29 trials with dietary supplements only three assessed food intake in patients.<sup>11</sup> Another limitation was absence of controlling for concentration of at least one the evaluated components of the TDS, so the compliance issues were controlled only using the CTDS. The absence of baseline serum testing for testosterone, FSH, and LH levels precluded complete evaluation for "pure" idiopathic infertility and served a certain bias for underestimation of endocrine factor.

In conclusion, the findings of our trial support the efficacy and safety of the combination of L-carnitine and L-acetyl-carnitine with L-arginine, glutathione, selenium, co-enzyme Q10, zinc, folic acid, and cyanocobalamin in improving sperm quality and increasing fertility rates in couples with a male factor of infertility. Based on the results obtained, mechanisms of interplay between the specific nutrients should be elucidated.

### CONFLICT OF INTEREST

Roman Kopets, Ivan Kuibida, Iryna Chernyavska, Vasyl Cherepanyn, Roman Mazo, Vitaliy Fedevych received personal fees for clinical visits, data collection; Sergiy Gerasymov received financial support for methodology, project administration, supervision, data analysis, manuscript preparation.

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