

ARCHIVIO ITALIANO DI UROLOGIA E ANDROLOGIA

ARCH ITAL UROL ANDROL

ARCHIVES OF ITALIAN UROLOGY AND ANDROLOGY

Vol. 84; n. 3, September 2012

Indexed in: Medline/Index Medicus, EMBASE/Excerpta Medica, Medbase/Current Opinion, SIIC Data Base, SCOPUS

Urological complications following inguinal hernioplasty

Gaetano Gulino, Michele Antonucci, Giuseppe Palermo, Francesco Sasso, Daniele Tienforti, Alessandro D'Addressi, Pier Francesco Bassi

Prostate cancer: What are the news in hormonal therapy? The role of GnRH antagonists

Filiberto Zattoni

Observational Database *Serenoa Repens* (DOSSER): Overview, analysis and results

Alessandro Bertaccini, Marco Giampaoli, Riccardo Cividini, Gian Luca Gattoni, Roberto Sanseverino, Tommaso Realonso, Giorgio Napodano, Andrea Fandella, Elisa Guidoni, Domenico Prezioso, Raffaele Galasso, Carmine Cicalese, Vincenzo Scattoni, Angelo Armenio, Giario Conti, Matteo Corinti, Roberta Spasciani, Giovanni Liguori, Nikolitsa Lampropoulou, Giuseppe Martorana

Problematics of open prostatectomy in an Ivorian District Hospital setting

Anthony Chukwura Mgbakor

Comparison of TVT, TVT-0/TOT and mini slings for the treatment of female stress urinary incontinence: 30 months follow up in 531 patients

Charalambous Stavros, Vouros Ioannis, Sakalis I. Vasileios, Anastasia Ch. Gkotsi, Salpiggidis Georgios, Athanasios Papathanasiou, Vasileios Rombis

Prospective open-label study on the efficacy and tolerability of a combination of nutritional supplements in primary infertile patients with idiopathic astenoteratozoospermia

Gian Maria Busetto, Aleardo Koverech, Masa Messano, Gabriele Antonini, Ettore De Berardinis, Vincenzo Gentile

Pre-treatment and post-treatment fertility in young male patients affected by Hodgkin and non-Hodgkin lymphoma

Nicolò de Luyk, Gabriele Pozzato, Giuseppe Ricci, Paolo Tamaro, Massimo Manno, Francesco Tomei, Carlo Trombetta

Nephron-sparing surgery for giant angiomyolipomas of kidney

Giampaolo Siena, Andrea Minervini, Agostino Tuccio, Gianni Vittori, Matteo Salvi, Arcangelo Sebastianelli, Omar Saleh, Alberto Lapini, Sergio Serni, Marco Carini

The role of the elastography in the diagnosis of prostate cancer: A retrospective study on 460 patients

Emilio Pozzi, Guglielmo Mantica, Christian Gastaldi, Marco Berardinelli, Dimitrios Choussos, Carlo Maria Bianchi, Alberto Roggia

Role of urethral constrictor in male urinary incontinence after surgery

Luciano De Giovanni, Marco Stefanucci, Paolo Menchinelli

Hemangiopericytoma of kidney: Case report and review of the literature

Muhsin Balaban, Cihangir Cetinel, Rahim Horuz, Cemal Goktas, Onder Canguven

The potential role of transrectal ultrasound as a tool for diagnosis or recurrence detection in bladder cancer. Two cases report

Andrea Fabiani, Alessandra Filosa, Mara Piergallina, Lucilla Servi, Gabriele Mammana

A case of angiomyolipoma of the spermatic cord and testicle

Roberto Giulianelli, Luca Albanesi, Francesco Attisani, Stefano Brunori, Barbara Cristina Gentile, Luca Mavilla, Gabriella Mirabile, Francesco Pisanti, Giorgio Vincenti, Teuta Shestani, Manlio Schettini

Neoadjuvant chemotherapy for invasive bladder cancer: An interesting case report

Davide Abed El Rahman, Victor Matei Deliu, Gennaro Musi, Danilo Bottero, Antonio Brescia, Giacomo Galasso, Federica Mazzoleni, Ottavio De Cobelli

Robot-assisted laproscopic removal of perirenal lymphangioma: A case report

Marco Finamanti, Alessandro Antonelli, Lorenzo Gatti, Angelo Peroni, Claudio Simeone

Realtime-elastostonography of the penis in patients with Peyronie's disease

Valentina Riversi, Valeria Tallis, Stefano Trovatelli, Arben Belba, Luca Volterrani, Francesca Iacoponi, Roberto Ponchietti

Official Journal
of the SIEUN



Società
Italiana di
Ecografia
Urologica
Andrologica
Nefrologica

Official Journal
of the SIURo



Società Italiana di
Urologia Oncologica

Official Journal
of the UrOP



Urologi
Ospedalità
Gestione Privata



Prospective open-label study on the efficacy and tolerability of a combination of nutritional supplements in primary infertile patients with idiopathic astenoteratozoospermia

Gian Maria Busetto¹, Aleardo Koverech², Masa Messano², Gabriele Antonini¹, Ettore De Berardinis¹, Vincenzo Gentile¹

¹ Sapienza Rome University, Policlinico Umberto I, Rome, Italy;

² Sigma-Tau, Nutraceuticals and Neglected Disease Department, Rome, Italy.

Summary

Aim of the study: To evaluate with an open-label study the efficacy and safety of a complex of nutritional supplements with antioxidant activity (L-carnitine, acetyl-L-carnitine, fructose, citric acid, selenium, coenzyme Q10, zinc, ascorbic acid, cyanocobalamin, folic acid) in primary infertile patients with idiopathic astenoteratozoospermia.

Methods: The study was conducted in a population of 114 infertile men (96 completed the study) diagnosed with idiopathic astenoteratozoospermia since at least 18 months.

Patients orally received a formulation (Proxeed - Sigma-Tau) containing L-carnitine 145 mg, acetyl-L-carnitine 64 mg, fructose 250 mg, citric acid 50 mg, selenium 50 mcg, coenzyme Q10 20 mg, zinc 10 mg, ascorbic acid 90 mg, cyanocobalamin 1.5 mcg, folic acid 200 mcg in combination once a day for 4 months.

Results: At the end of study, the mean sperm progressive motility showed a statistically significant increase from 18.3 ± 3.8 to 42.1 ± 5.5 . Sixteen patients achieved pregnancy during the study. No significant improvement were observed for sperm density and rate of morphologically normal forms. The treatment was well tolerated.

Conclusions: Carnitines in association with others functional substances can improve the most important parameters of sperm quality.

KEY WORDS: Astenoteratozoospermia; Male infertility, Sperm quality.

Submitted 31 May 2012; Accepted 15 June 2012

INTRODUCTION

According to data reported by the *Istituto Superiore di Sanità* (ISS), that are similar to *National Institute of Health* (NIH) ones, infertility affects about 15% of couples. Several causes can lead to infertility. The data collected by the *National Register on Medically Assisted Reproduction* were:

- male infertility (35.4%),
- female infertility (35.5%),
- male and female infertility (15%),
- idiopathic infertility (13.2%),
- other (1%).

In recent years, male fertility was significantly reduced. According to many studies, the mean sperm count per

ml would have almost halved over the last 50 years (7). Male infertility certainly involves an important social component: in addition to individual risk factors, also environmental conditions and lifestyle (including stress) appear to influence. Cigarette smoke, that harms semen (smokers often have an higher rate of spermatozoa with abnormal morphology), some work conditions that expose to radiation, toxic substances or micro-trauma increase the risk of infertility (6).

A series of experimental evidences has shown a causal relationship between oxidative stress and sperm deterioration (14).

The extent of oxidative stress is defined by the ROS/TAC

score, that is the balance between oxidative radicals (ROS) and total antioxidant capacity (TAC), dependent on the endogenous scavenger factor production. The damage caused by an altered ROS/TAC score on the structure and function of spermatozoa, according to a series of relevant reports in the literature, seems to affect all sperm parameters, but particularly motility and morphology (3, 9). Based on these evidences, there is a rising need of therapeutic interventions to reduce oxidative damage by improving the score ROS/TAC. Antioxidant treatments achieved significant results in order to improve sperm quality in patients with dyspermia secondary to varicocele or prostatic inflammation (2). The role of carnitine, fructose and many other substances has been evaluated in many studies (1, 7, 16) showing a contribution in the processes of regulation and optimization of sperm function, although some aspects of their activities have to be further defined. Therefore, a pharmacological modulation of an unfavorable microenvironment could and should act on different biological networks to achieve overall superior results. Based on this background, this study reports the results from a prospective open-label evaluation of the efficacy and tolerability of an antioxidant supplements combination (L-carnitine, acetyl-L-carnitine, fructose, citric acid, selenium, coenzyme Q10, zinc, ascorbic acid, cyanocobalamin, folic acid) (*Proxeed NF[®] Sigma-Tau*) to improve sperm parameters in men with idiopathic infertility. This formulation aims to support different functional metabolic mechanisms: enhancement and protection of energy metabolism, protection from excessive oxidative stress (ROS) and modulation of sperm biological and cytoplasmic responses. Energy metabolism is in particular supported by carnitine, fructose and citric acid. The carnitine system has been under evaluation for a long time: its cofactors are essential for the transfer of lipids to the mitochondria and thus for energy production and are also involved in the removal of medium and small chain fatty acids, that tend to accumulate as a result of the metabolism. Moreover, although not having the typical structure of an antioxidant molecule, some derivatives of carnitine (such as acetyl- or propionyl-carnitine) seem able to contrast lipid oxidation by oxidative stress and thus preserve the cytological structure of spermatozoa and prevent apoptosis. All the mechanisms of their activities are not yet known, but these characteristics make these molecules particularly useful in tissues, where there is a very active lipid metabolism, as in epididymal spermatozoa. Moreover, the metabolic relevance of carnitines is prompted by their concentration in the epididymal sperm, being higher than any other district in the body. The formulation also contains coenzyme Q10, vitamin C and selenium that demonstrate to have a protective role against excessive oxidative stress. The activity of this formulation is completed by the biological action of positive modulators as zinc, folic acid, vitamin B12 that play a role in optimizing fertility and that can be enbalanced or deficient in many cases of hypofertility.

MATERIALS AND METHODS

One-hundred fourteen patients diagnosed with primary idiopathic infertility and astenoteratozoospermia by at

least 18 months (mean age 31 years; range 21-46 years) were enrolled. All the patients underwent diagnostic evaluation including medical history, physical examination, semen analysis with MAR-test (semen analyses were performed for each patient at the beginning, during and at the end of the study), sperm and urethral smear culture for *Mycoplasma* and *Chlamydia*, blood counts and basic blood chemistry assay, measurement of plasma testosterone, E2, LH, FSH and prolactin, testicular ultrasound and Doppler. Selection criteria used for enrollment are listed below:

Inclusion criteria

- infertility by at least 18 months
- sperm volume > 1 ml
- sperm density < 20 million/ml
- progressive motility (a + b) < 50%
- percentage of morphologically normal forms (WHO sec.) < 50%
- white blood cells in semen < 1 million/ml
- culture tests negative
- hormonal levels within normal limits.

Exclusion criteria

- testis primary diseases (cryptorchidism or atrophy secondary to orchitis, trauma, torsion, exposure to chemical or physical agents and previous surgery)
- evidence of autoimmune disease
- endocrine and metabolic diseases
- use of drugs or toxic substances jeopardizing reproductive function.

TREATMENT AND EVALUATION PARAMETERS

Patients received orally a formulation containing L-carnitine 145 mg, acetyl-L-carnitine 64 mg, fructose 250 mg, citric acid 50 mg, selenium 50 mcg, coenzyme Q10 20 mg, zinc 10 mg, ascorbic acid 90 mg, cyanocobalamin 1.5 mcg and folic acid 200 mcg once a day for 4 months after providing informed consent for the study. Efficacy and tolerability assessment was carried out by office checkup, blood count and basic blood chemistry assays and semen analysis in the end of treatment. Sperm concentration, motility and morphology were evaluated according to WHO criteria. Primary objectives of the study were the evaluation of efficacy (in terms of average increase in the rate of progressive motility and morphologically normal forms according to WHO criteria) and tolerability based on the medical history and laboratory. Secondary objective was the evaluation of mean increase in sperm density and pregnancy incidence during treatment. Results are shown as mean \pm SD. Statistical significance was evaluated by Student t-test.

RESULTS

Ninety-six patients completed the study. Sixteen patients (14%) dropped out after achieving spontaneous pregnancy during treatment. Statistical data, showing an increase in vitality of spermatozoa and their concentration, correlate the effects of treatment with pregnancies. Two patients were not available at the final follow-up. The

Table 1.

	Before treatment	After treatment	P value
Patients, n.	96	80*	
Pregnancy, n. (%)	0 (0%)	17 (15%)*	
Progressive sperm motility, %	18.3 ± 3.8	42.1 ± 5.5	< 0.05
Morphologically normal forms, %	32.8% ± 7.1	38.4 ± 6.2	NS

* 16 patients (14%) dropped out after achieving spontaneous pregnancy during treatment.

mean progressive sperm motility was 18.3 ± 3.8 at baseline and 42.1 ± 5.5 after treatment, showing a statistically significant difference ($p < 0.05$), with not significant increase in the rate of morphologically normal forms (from $32.8\% \pm 7.1$ to 38.4 ± 6.2). Seventeen patients (15%) obtained spontaneous pregnancy during treatment, with only one of them completing the study. With regard to tolerability, neither adverse events nor abnormal blood chemistry values due to therapy were detected during treatment. In conclusion, the association of L-carnitine and acetyl L-carnitine (*Proxeed - Sigma-Tau*) is an effective adjuvant treatment for idiopathic asthenospermia, being able to increase semen quality and also becoming an important support in view of a possible artificial insemination.

DISCUSSION

Spermatozoa, like other cells in the body can be impaired by ROS. An aspect that must be considered in the evaluation of sperm wellness preservation is the influence of ROS. As any harmful agent, ROS can affect some typical parameters that, generally, are evaluated as index of sperm quality. These parameters are physical-chemical and morphological. The former include colour, appearance, volume, pH, viscosity and fluidity of the ejaculate. The latter include morphological alterations of sperm forms and motility, as most important parameter. In sperm evaluation of paramount importance are also the concentration rate and the total number of sperm cells in the ejaculate.

Particularly, is well established the correlation between increase of ROS and ageing, and between ageing and loss of reproductive quality of ejaculate (11). This is not the only explanation for infertility but surely has a strong impact on the reproductive capacity of every man for all his lifespan. The onset of genetic deterioration of sperm is believed to begin around the age of thirty.

It is possible for men to father children in old age, but the genetic quality of sperm, as well as its volume and motility, typically decrease with age (11). The sperm of older people is less likely to result in a successful pregnancy and, moreover, the cumulative fragmentation of sperm DNA over time makes it more likely that an older father will transmit a genetic disease.

A metabolic approach can be really useful to handle male infertility. The present experimental study evaluated the utilization of functional substances (*Proxeed*) to improve sperm motility, concentration and morphology, but also the increase in sperm density and pregnancy incidence during treatment. *Proxeed* is a patented for-

mulation to improve sperm quality and increase the chance of conception, that includes L-carnitine and L-acetyl-carnitine, as main component of its formulation. This dietary supplement has demonstrated its effectiveness to enhance and protect energy metabolism, protect tissues and especially sperm from free radical injury and enhance the cytoplasmic and biological response of the sperm. All these effects are related to the presence of the carnitines that enhance mitochondrial fatty acid beta oxidation, an important pathway for the energy production (4). Carnitines are strong antioxidant that can protect against ROS damage (2).

In our study, at the end of the experimental treatment we observed a marked increase in quality parameters of sperm like motility (from 18.3 to 42.1). The morphological parameters were not influenced by the administration of *Proxeed* formulation, but the 15% of patients spontaneously obtained pregnancy during treatment.

The safety of the formulation is assured by its composition of vitamins, natural elements and enzyme cofactors. Tolerability was confirmed by the total absence of adverse effects during the four months of treatment in all the ninety-six patients in the study.

On this basis it can be established that the use of carnitines, and other functional substances like enzyme cofactors, natural elements and vitamins can be an efficacious strategy to handle male infertility. In fact carnitines in association with other functional substances included in this formulation can improve the parameters of sperm quality (Table 1).

ACKNOWLEDGEMENTS

Authors thank Dr Luigi Pinto for his help in preparing this manuscript.

REFERENCES

1. Agarwal A, Nallella KP, Allamaneni SS, Said TM. Role of antioxidants in treatment of male infertility: an overview of the literature. *Reprod Biomed Online* 2004; 6:616-27.
2. Arduini A. Carnitine and its acyl esters as secondary antioxidants? *Am Heart J* 1992; 6:1726-7.
3. Balercia G, Regoli F, Armeni T, et al. Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. *Fertil Steril* 2005; 3:662-71.
4. Bremer J. Carnitine: metabolism and functions. *Physiol Rev* 1983; 4:1420-80.
5. Cavallini G, Ferraretti AP, Gianaroli L, et al. Cinnoxycam and L-

carnitine/acetyl-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. *J Androl* 2004; 5:761-70; discussion 771-2.

6. Costa M, Canale D, Filicori M, et al. L-carnitine in idiopathic asthenozoospermia: a multicenter study. *Italian Study Group on Carnitine and Male Infertility. Andrologia* 1994; 3:155-9.

7. Lenzi A, Sgrò P, Salacone P, et al. A placebo-controlled double-blind randomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia. *Fertil Steril* 2004; 6:1578-84.

8. Lenzi A, Lombardo F, Sgrò P, et al. Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. *Fertil Steril* 2003; 2:292-300.

9. Moustafa MH, Sharma RK, Thornton J, et al. Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. *Hum Reprod* 2004; 1:129-38.

10. Pacey AA. Environmental and lifestyle factors associated with sperm DNA damage. *Hum Fertil (Camb)*. 2010; 4:189-93.

11. Schwartz D, Mayaux MJ, Spira A, Moscato ML, Jouannet P, Czyglik F, David G. Semen characteristics as a function of age in 833 fertile men. *Fertil Steril* 1983; 4:530-5.

12. Sigman M, Glass S, Campagnone J, Pryor JL. Carnitine for the treatment of idiopathic asthenospermia: a randomized, double-blind, placebo-controlled trial. *Fertil Steril* 2006; 5:1409-14.

13. Syntin P, Robaire B. Sperm structural and motility changes during aging in the Brown Norway rat. *J Androl*. 2001; 2:235-44.

14. Smith R, Kaune H, Parodi D, et al. Extent of sperm DNA damage in spermatozoa from men examined for infertility. Relationship with oxidative stress. *Rev Med Chil* 2007; 3:279-86.

15. Vicari E, Calogero AE. Effects of treatment with carnitines in infertile patients with prostatic-vesiculo-epididymitis. *Hum Reprod* 2001; 11:2338-42.

16. Zhou X, Liu F, Zhai S. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. *Asia Pac J Clin Nutr* 2007; 16:383-90.

Correspondence

Gian Maria Busetto, MD
Sapienza Rome University
Policlinico Umberto I - Department of Urology
Viale del Policlinico 155 - 00161 Rome, Italy
gianmaria.busetto@uniroma1.it

Aleardo Koverech, MD
Masa Messano, MD
Nutriceuticals and Neglected Disease Department,
Sigma-Tau - Rome, Italy

Gabriele Antonini, MD
Ettore De Berardinis, MD
Vincenzo Gentile, MD
Sapienza Rome University
Policlinico Umberto I - Department of Urology
Viale del Policlinico 155 - 00161 Rome, Italy