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ORIGINAL ARTICLE

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# May antioxidant therapy improve sperm parameters of men with persistent oligospermia after retrograde embolization for varicocele?

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**Abstract** We performed a randomized, prospective, controlled, intention to treat study in order to determine the effectiveness of an antioxidant therapy in improve the quality of seminal fluid parameters and the natural pregnancies in men with persistent oligospermia (5–20 million/ml) 6 months after retrograde embolization. Forty-two subjects were enrolled and randomized in the study. Treated group (20 subjects) was assigned to receive antioxidant therapy (NAC 600 mg and vitamins–minerals). Untreated group (22 subjects) received no adjunctive medical therapy and was used as controls. Our data were analyzed with an intention to treat strategy. A statistically significant increase in sperm count after antioxidant therapy was recorded ($P = 0.009$). After this therapy, no statistical differences in percentage of WHO class A motile sperm ($P = 0.752$) and typical forms ($P = 0.926$) were found. The univariate logistic regression analysis showed that a man treated with antioxidant therapy presented a probability to have a normal sperm count 20-fold (OR = 20.1; CI 95% = 1.05–43.2; $P = 0.014$) higher than a man who was untreated. No significant impact on spontaneous pregnancies was found after antioxidant therapy. Despite this preliminary data, we show that antioxidant therapy based on a combination of NAC and micronutrient supplementation can be helpful in improve the sperm count at least in a subset of oligospermic males. However, this improving in sperm count is not associated with a significant increase in spontaneous pregnancies after 12 months.

**Keywords** *N*-acetylcysteine (NAC) · Antioxidants · Vitamins · Micronutrients · Oxidative stress · Reactive oxygen species (ROS) · Spermatogenesis · Fertility · Infertility · Varicocele

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## Introduction

Oxidative stress has been proposed to have a causal/concausal role in many cases of male infertility, independently of the underlying pathology [1]. In testis and in seminal fluid, reactive oxygen species (ROS) are produced by spermatogenic cells, spermatozoa, immune cells, mainly neuthrophils, and high amounts of them can be produced by immature and abnormal spermatozoa and during inflammation [2]. In seminiferous tubules oxidative stress can produce both excessive death of spermatogenic cells and nonlethal germ cell damages [3–6]. Moreover, mature spermatozoa are highly susceptible to lipid peroxidation, and largely dependent on the antioxidant defences present in seminal plasma [7].

The concentration of ROS's in seminal fluid is inversely related to sperm quality and a reduced total antioxidant capacity and/or increased ROS production can significantly contribute to the physiopathology of male infertility [8–10]. In particular, deficits of glutathione (GSH), GSH-dependent glutathione peroxidases, the antioxidant vitamin E,

Vitamin C and coenzyme Q10, have been found to be associated with sperm quality alterations [11–14]. Remarkably, it has been reported that oligoasthenospermic patients have high ROS/low antioxidant levels not only in seminal fluid but also in blood plasma [15], and reduced levels of omega-3 polyunsaturated fatty acids (PUFA) in spermatozoal membranes, blood plasma and erythrocyte membranes [15]. Varicocele is a pathology frequently associated with sperm quality alterations and infertility and acts together with other genetic/molecular predisposing factors to the genesis of male infertility [16]. Varicocelectomy can reduce oxidative stress and improve sperm parameters in a significant percentage of patients with oligoasthenospermia, but it is not effective in all cases [17]. A pharmacological approach can further improve sperm quality, and can be of particular relevance in patients with little or no improvement after surgical treatment. However, despite the empirical use of many drugs, only a few molecules have been proven to be really useful in selected cases of dyspermia, and the overall efficacy of the pharmacological approach is actually modest.

Moreover, a sovranutritional intake of antioxidants (antioxidant therapy) has been demonstrated to be effective in reducing oxidative stress and improving sperm parameters, in particular motility and morphology, both in men and animal models [18]. However, it is still not clear which antioxidants/micronutrients or combinations of these can be effective in specific patients, and clear dose-effect correlations are lacking. As a consequence, there are still no well-defined antioxidant/micronutrients-based therapeutic protocols for male infertility. Starting from the evidence present in the literature, we have evaluated the clinical efficacy of a combination of NAC (a powerful antioxidant that increases GSH synthesis) and a number of micronutrients, on selected cases of men who presented oligospermia (5–20 million/ml) 6 months after a surgical treatment with retrograde embolization.

## Materials and methods

### Patients recruitment

From January 2003 to June 2005 all men who had performed a retrograde embolization for a color Doppler ultrasound grade 3, 4, and 5 varicocele with concomitant oligospermia were considered for this study. Of these, were enrolled only subjects who had persistent oligospermia 6 months after retrograde embolization. A state of oligospermia was defined as a reduction in sperm density (sperm count less than 20 million/ml) whose chief complaint was primary couple infertility longer than 12 months. Subjects with smoking habit and alcohol consumption, treatment with drugs potentially interfering with spermatogenesis (e.g., antiblastics, antibiotics like fenothiazines, metoclopramide, cimetidine, psychoactive drugs) taken within the 3 months before study entry, any serious medical or psychiatric condition or illness that would not permit the patient to be managed according to the protocol, abnormal hormonal profile, sperm infections (white blood cells greater than 1 million white blood cells/ml semen) were excluded. Before retrograde embolization, all subjects were evaluated with a detailed history, physical and genital examination, hormonal determinations (FSH concentrations, testosterone level free and total, luteinizing hormone level, prolactin level) and prostate, seminal vesicles, ejaculatory ducts and scrotal ultrasound. The scrotal contents were examined with patients in a standing position. Volume, position, and consistency of testes and epididymes as well as presence of vasa deferentia were noted. Palpation of the pampiniform plexus was done with and without the patients performing a Valsalva maneuver. The clinical diagnosis of varicocele was confirmed by color Doppler technique with a high definition device and with a 10 MHz linear probe. Varicocele was classified according to the severity of venous reflux and defined as a venous reflux longer than 2 s at the level of pampiniform plexus with or without the Valsalva manoeuvre. The severity of venous reflux was classified in grade 1 (venous reflux with Valsalva manoeuvre limited to the cranial portion of the cord), grade 2 (venous reflux with Valsalva manoeuvre until the upper pole of the testicle), grade 3 (venous reflux with Valsalva manoeuvre until the lower pole of the testicle) and grade 4 (reflux in basal conditions increased by Valsalva) and grade 5 (reflux in basal conditions which does not increase with Valsalva) [19].

### Experimental design, randomization and treatment

Subjects were randomly assigned to either antioxidant therapy or no medical therapy. Randomization number was assigned by random allocation software using a block randomization design. A written informed consent was obtained before study entry. All steps of randomization process were performed blindly in the pharmacy of our hospital. The drug administration was started the day after the randomization and was continued for a maximum of 90 days or after withdrawal of consent. A total of 78 patients matched all the selected criteria. Of these 78 eligible men, 24 declined to participate in the study and a total of 42 subjects were enrolled; 20 patients (treated group) were assigned to receive antioxidant therapy once a day. The other 22 patients (control group) received no antioxidant therapy and were used as controls. In the present study, we utilized as antioxidants therapy a commercial preparation of NAC (NAC 600 mg) and vitamins–minerals. Patients in the treated group consumed daily the following



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elements: NAC 10 mg/kg/die, Vit C 3 mg/kg/die, Vit E 0.2 mg/kg/die, Vit A 0.06 IU/kg/die, thiamine 0.4 mg/kg/die, riboflavin 0.1 mg/kg/die, piridoxin 0.2 mg/kg/die, nicotinamide 1 mg/kg/die, pantothenate 0.2 mg/kg/die, biotin 0.04 mg/kg/die, cyanocobalamin 0.1 mg/kg/die, ergocalciferol 8 IU/kg/die, calcium 1 mg/kg/die, magnesium 0.35 mg/kg/die, phosphate 0.45 mg/kg/die, iron 0.2 mg/kg/die, manganese 0.01 mg/kg/die, copper 0.02 mg/kg/die, zinc 0.01 mg/kg/die. Follow-up included clinical examination with the evaluation of adverse events (AEs) and the evaluation of spontaneous pregnancies every 4 weeks until 12 months after treatment withdrawal.

### Seminal fluid analysis

Seminal fluid was obtained after 5 days of sexual abstinence. Semen samples were obtained by masturbation. Ejaculate analysis was performed according to WHO guidelines (WHO, 1999) and included physical parameters (ejaculate volume, pH, color) as well as spermatozoa concentration, motility and morphology (Papanicolaou staining). All ejaculate analysis was analyzed blindly with respect to the treatment groups.

### Study endpoints

For the evaluation of antioxidant therapy efficacy three seminal fluid parameters were chosen: sperm count (millions/ml), percentage of WHO class A motile sperm and percentage of typical forms. As secondary endpoint we assessed the natural pregnancies achieved during the observation period of 12 months after antioxidant therapy.

### Statistical analysis

In order to detect a difference of 30% or more between the 2 groups in the effect size (two-side type I error of 5% and type II error of 0.2%) 42 subjects were necessary. All patients who received at least one dose of the drug were included in the analysis and an intention-to-treat strategy was applied. Statistical analysis was performed using SPSS 12.0.1 (SPSS, Inc., Chicago, IL, USA) software. All $P$ values less than 0.05 were considered to indicate significance. All statistical tests were two-tailed. Because our study variables were not normally distributed (Shapiro–Wilk test $P < 0.05$) continuous variables were presented as medians and interquartile range (25th and 75th percentile) and analyzed with Wilcoxon–Mann Whitney Rank Sum Test. Categorical variables between groups were compared with Chi-square test or Fisher's exact test when the tables were too sparse. Logistic regression analysis was performed to determine the strength of relationship between antioxidant therapy and seminal fluid parameters.

## Results

The randomization of the first patients occurred in January 2003 and the last patient completed 12 months' follow-up in April 2006. A total of 42 patients were enrolled in the study. The participation rate was 53.8% (42/78 subjects) and all randomized patients completed the protocol treatment and were analyzed. In the Table 1 we summarize demographic characteristics of all patients. No statistical difference between groups in age ($P = 0.80$), partner age ($P = 0.98$) and grade III ($P = 0.836$), IV ($P = 0.259$) and V ($P = 0.231$) was found. However, a significant difference in the duration of infertility ($P = 0.019$) was observed. Among the different seminal fluid parameters, a significant difference in sperm count in treated group was found (Table 2). In particular seminal fluid analysis showed that the median value of sperm count was 14.42 (11.75–15.45) millions/ml before treatment and 32.58 (18.75–35.25) millions/ml after antioxidant treatment ($P = 0.009$). The percentage of WHO class A motile sperm and the percentage of typical forms were 60 and 23.4% before treatment, and 58 and 29.7% after antioxidant treatment, respectively. In this group, no statistical differences were found in the percentage of WHO class A motile sperm ($P = 0.847$) and in the percentage of typical forms ($P = 0.926$). In the control group no statistical differences were found with respect to all seminal fluid parameters at baseline and after three months. In particular the median values of sperm count were 12.29 (10.75–13.45) millions/ml at baseline and 15.00 (13.75–16.35) millions/ml after 90 days ($P = 0.1$). In this group, the percentage of WHO class A motile sperm and the

**Table 1** Demographic and clinical parameters of infertile men

| | Control | Treated | $P$ values |
|---|---|---|---|
| Age (years) | 33(23–36) | 32(27.5–35.5) | 0.80[b] |
| Partner age (years) | 27(23–36) | 33(22–36.5) | 0.98[b] |
| Duration of infertility (months) | 20(16–25) | 24(21–39.5) | 0.019[b] |
| Grade III varicoceles | 7/22(32%) | 6/20(30%) | 0.836[a] |
| Grade IV varicoceles | 9/22(41%) | 4/20(20%) | 0.259[a] |
| Grade V varicoceles | 6/22(27%) | 10/20(50%) | 0.231[a] |

[a] $\chi^2$ corrected test or Fisher's Exact test when appropriate

[b] Wilcoxon–Mann Whitney Rank Sum Test



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**Table 2** Sperm quality and pregnancies rate of infertile men before and after antioxidant therapy

| | Control | | | Treated | | |
|---|---|---|---|---|---|---|
| | Baseline | Re-assessment[a] | *P* values | Baseline | Re-assessment[a] | *P* values |
| Number[b] (million/ml) | 12.29 (10.75–13.45) | 15.00 (13.75–16.35) | 0.1[c] | 14.42 (11.75–15.45) | 32.58 (18.75–35.25) | 0.009[c] |
| WHO class A Motile sperm (%) | 56% | 51% | 0.976[d] | 60% | 58% | 0.847[d] |
| Typical forms (%) | 20.3% | 22.4% | 0.833[d] | 23.4% | 29.7% | 0.926[d] |

| | Treated | Control | *P* values[d] | OR[e] | 95% CI |
|---|---|---|---|---|---|
| Sperm count ≥ 20 million/ml | 6/20 (30%) | 0/22 (0%) | 0.014 | 20.1 | 1.05–43.2 |
| Sperm count < 20 million/ml | 14/20 (70%) | 22/22 (100%) | | | |
| Spontaneous pregnancies | 1/20 (5%) | 0/22 (0%) | 0.95 | | |
| pregnancies | 19/20 (95%) | 22/22 (100%) | | | |

[a] Performed 3 months after therapy

[b] Median values and 25th–75th percentile

[c] Wilcoxon–Mann Whitney Rank Sum Test

[d] $\chi^2$ Corrected test or Fisher's Exact test when appropriate

[e] Logistic regression

percentage of typical forms were 56 and 20.3% at baseline, and 51 and 22.4% after 90 days, respectively, with no statistical difference (Table 2). After antioxidant treatment 6 out of 20 subjects (30%) in the treated group presented a sperm count greater than 20 millions/ml, while no man in the control group had a sperm count greater than this value. The univariate logistic regression analysis showed that a man treated with antioxidant therapy presented a probability to have a normal sperm count 20.1-fold (OR = 20.1; CI 95% = 1.05–43.2; $P = 0.014$) higher than a man who was untreated (Table 2). Twelve months after discontinuation of therapy one patient among those who had normal sperm count after antioxidant therapy impregnated his partner. None of the men's partners in the control group achieved a spontaneous pregnancy. No significant AEs imputable to antioxidant therapy were recorded.

## Discussion

Varicocele has been associated with the presence of oxidative stress in seminal fluid, and both the levels of oxidative stress and of sperm damage seem to be related to the grade of varicocele [20]. However, in some patients surgical treatment is not effective in restoring both normal seminal parameters and a natural fertility [21]. Only a few pharmacological treatments have been demonstrated to be really helpful in these cases [4].

In this paper we describe the results of a prospective, controlled, randomized, intention-to-treat study. We selected a specific subpopulation who presented oligospermia (5–20 million/ml) 6 months after retrograde embolization for varicocele. We showed that an oral antioxidant therapy, based on NAC and micronutrient elements and administered for 90 days, induced a significant improvement in sperm count. No significant improvement was observed in the percentage of WHO class A motile sperm and of typical forms. These data are partially in agreement with other studies but the entity of our sperm count improvement is very wide. Our results could be explained considering that the selected population presented a basal sperm count near to the normality range, and this might suggest that the spermatogenesis might not be heavily damaged. In addition, although in these men there was a persistence of oligospermia after surgical treatment, it has been shown that surgical treatment alone seems to influence the regulation of the antioxidant defense system in patients with varicocele, and this in turn, seems to influence the sperm quality [22]. In these subpopulations the administration of an antioxidant therapy might have influenced, with an additive mechanism, the antioxidant defense system regulation favoring the improvement of sperm quality. Additionally, after medical treatment, six men presented a sperm count greater than 20 million/ml. Individuals performing antioxidant treatment presented 20.1-fold (OR = 20.1; CI95% = 1.05–43.2; $P = 0.014$) more likely to have a normal range sperm count than individuals who did not perform this therapy. However, the evident increment in the number of men with a normal sperm count was not associated with a significant increase of spontaneous pregnancies after 12 months even if the only man who impregnated their partner presented a normal sperm count after antioxidant therapy. These data about spontaneous pregnancies emphasized that the different treatment modalities are more



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efficacious in terms of increasing the cumulative pregnancy rates among men with lower sperm concentrations than among those with only moderate oligospermia (e.g., >10 million/ml) [23]. In the latter, it seems to be the functional impairment of spermatozoa rather than their number that is responsible for the decreased fertility. Given the complex nature of redox regulation, it is unlikely that the assumption of a single antioxidant, i.e., only a part of big molecular machinery, can produce more than a limited antioxidant effect, unless there is a specific deficit [24]. On the basis of the following considerations, we decided to evaluate the effect of a cocktail rather than the effect of single antioxidants/micronutrients on the improvement of sperm parameters. The chose of our cocktails born from the evidences that cystein is an essential element in redox regulation, as a direct ROS scavenger and as a component of GSH and redox cystein-rich proteins [25]. Moreover, in addition to redox regulation, adequate cystein availability to the germ cells is essential for the synthesis of cystein rich proteins during spermatogenesis [26]. Thus, we used NAC as the main molecule in our study since it is a cell permeant form of cystein, and can be assumed orally, whereas GSH requires intramuscular administration [27]. For these reasons, we also decided to add other antioxidants/micronutrients, both to obtain the effect of a moderate over-nutritional intake, and to cover potential nutritional deficits. The duration of the treatment (90 days) was established taking into account the duration of a full spermatogenetic cycle (around 72 days). We recognized potential limitations in our study as the relative low number of patients enrolled and the realization of a no-placebo study. The use of a placebo in clinical research continues to be a topic of debate in the medical community. We decided to not use placebo since we believe that it use might be not "fully" ethical couples especially when placebo might not increase the overall quality of trial. In this regard, the absence of a placebo arm is acceptable when there is reasonable confidence that the assessment of study end-points is objectives. We believe that our end-points are objectives and realistic for a trial of this type. In addition it is often possible to have a blinded evaluator that carry out endpoint assessment, even if the overall trail is not double blind.

## Conclusion

Despite this preliminary data, we show that antioxidant therapy based on NAC and micronutrient supplementation can be helpful in improve the sperm count at least in a subset of oligospermic males. However, the improving in sperm count was not associated with an increase in spontaneous pregnancies after 12 months. More efforts should be done in the direction of the definition of new pharmacological protocols based on antioxidants/micronutrients in order to improve sperm quality of infertile males.

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