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Combined treatment with myo-inositol, alpha-lipoic acid, folic acid and vitamins significantly improves sperm parameters of sub-fertile men: a multi-centric study

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Abstract. – OBJECTIVE: Reduction in motility and number of spermatozoa and change in their morphology are some of the most relevant causes of male infertility. Production of reactive oxygen species may affect motility, morphology and DNA stability of spermatozoa. This study aimed at evaluating the effect of combined treatment with myo-inositol, alpha-lipoic acid, folic acid, betaine and vitamins (namely, Sinopol®) on semen parameters of sub-fertile men.

PATIENTS AND METHODS: We recruited 143 sub-fertile men, 26-53 years aged, no-smokers, without any testicular pathologies, with a normal endocrinological/metabolic profile, and no concomitant consumption of drugs. Out of them, 25 patients did not meet study inclusion criteria mainly due to the history of genital diseases that came to light after Sinopol® prescription. Among the 118 men that fulfilled inclusion criteria, 10 (8.4%) patients were lost at follow-up and in 8 (6.8%) cases the partner got pregnant spontaneously. Thus, 100 patients completed the study and semen analysis was performed before and after 90 days of treatment.

RESULTS: Semen quality improved after 90 days of treatments, with a statistically significant increase of sperm concentration (p=0.0009), of number of spermatozoa (p=0.0017), of progressive motility (p=0.0047), of total motile sperm count (p=0.0010), and of normal sperm morphology (p<0.0001).

CONCLUSIONS: For the first time we reported that a combination of nutraceuticals composed of myo-inositol, alpha-lipoic acid, folic acid, betaine and vitamins improves sperm parameters in sub-fertile men. We are aware that to clarify the clinical relevance of the data studies with larger sample sizes and longer durations are needed, as well as evaluation of myo-inositol and alpha-lipoic acid co-treatment effectiveness in improving the chances to obtain a pregnancy spontaneously or following assisted reproduction.

Key Words:
Myo-inositol, Alpha-lipoic acid, Asthenozoospermia, Semen quality, Pregnancy.

Introduction

Infertility is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse[1]. It affects about 10% of couples of childbearing age. Male partner is the sole cause, or a contributing cause, of infertility in about 40% of infertile couples[2]. It has been estimated that almost 7% of men in their reproductive age are subfertile or infertile, due to testicular, pre-testicular or post-testicular problems. Conditions such as varicocele, cryptorchidism, and hypogonadism are among the many causes of male infertility[3]. Nevertheless, idiopathic infertility is found in about 25-30% of infertile patients[4]. Males with sperm parameters below the World Health Organization (WHO) reference values are considered to have male factor infertility[5]. The most significant of parameters are low sperm concentration (oligospermia), poor sperm motility (asthenospermia), and abnormal sperm morphology (teratospermia). Other factors less well associated with infertility include semen volume and seminal markers of epididymal, prostatic, and seminal vesicle function[6]. Some 30% to 80% of male factor subfertility cases are believed to be due to the damaging effects of

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oxidative stress that occurs when reactive oxygen species (ROS) overcome the semen's natural antioxidant defenses and cause cellular damage to the sperm[7]. ROS include a broad spectrum of molecules such as oxygen free radicals (superoxide anion, hydroxyl radical, hyperoxyl radical), non-radical species (hypochlorous acid and hydrogen peroxide), reactive nitrogen species and free nitrogen radicals (nitroxyl ion, nitrous oxide, peroxynitrite). Physiologically, in the seminal plasma homeostasis between free radicals and antioxidant substances is guaranteed by a very complex system involving superoxide dismutase, catalase and glutathione peroxidase dependent activity, associated with significant concentrations of antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E) and glutathione. Several conditions may alter this antioxidant system and potentially lead to a harmful accumulation of ROS in seminal fluid. These include pathological conditions involving the reproductive tract (varicocele, prostatitis), infections, autoimmunity and chronic disease, environmental factors (high temperatures, electromagnetic radiation, pesticides and air pollution), some lifestyles (smoking, alcohol abuse, drug addiction), and nutritional errors (unbalanced hyperlipidic diet, obesity, poor diet)[8]. Recently, metabolic syndrome and its individual symptoms as obesity, dyslipidemia, hypertension, and impaired glucose metabolism with insulin resistance, have been associated with detrimental effects on sperm fertility potential and DNA integrity[9]. In fact, research over the past decade has demonstrated that the pro-inflammatory state seen in metabolic dysfunction and the subsequent development of oxidative stress may have direct detrimental effects on normal spermatogenesis and sperm function[10]. Spermatozoa are particularly susceptible to oxidative damage because their plasma membranes are rich in polyunsaturated fatty acids and have low concentrations of scavenging enzymes[11]. Under physiologic states, spermatozoa themselves produce little amounts of ROS that are needed for capacitation and acrosomal reaction[12]. Excessive generation of ROS in semen by leukocytes as well as by abnormal spermatozoa may affect motility, morphology, mitochondrial membranes, chromatin condensation and DNA stability, thus compromising sperm function[13]. Oral supplementation with antioxidants may improve sperm quality by reducing oxidative stress[14], thus dietary supplementation with antioxidants has gained much attention in recent years[13,15-17]. One potent antioxidant is alpha-lipoic acid (ALA), a natural short-chain fatty acid containing sulfhydryl groups[18]. It is active both in the aqueous phase (cytoplasma) and in the lipid phase (membrane) of cells. ALA is the coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase mitochondrial respiratory enzymes and thus it improves mitochondrial function[19,20]. Inside cells and tissues, ALA is reduced to dihydrolipoic acid (DHLA), which has more antioxidant properties than does lipoic acid. Both DHLA and ALA have metal-chelating capacity and scavenge ROS, and DHLA is able to regenerate endogenous antioxidants vitamin E, vitamin C and glutathione, and to repair oxidative damage. Alpha-lipoic acid is also a main factor in the Krebs cycle and contributes to ATP biosynthesis, which is crucial for the sperm viability[21]. Results of animal studies showed that the percentage of motile sperm increased dramatically after ALA administration[21-23.] Until now, only one study has been conducted in humans[24] and, although further investigation is suggested in this regard, medical therapy of asthenoteratospermia with ALA supplement could improve quality of semen parameters. Moreover, ALA was shown to be effective in reducing symptoms of diabetic polyneuropathy[25] and body weight in obese subjects[26].

Few studies[27-31] have investigated the role of another possible antioxidant agent, namely myo-inositol (MYO), both for systemic treatment of male infertility and for improvement of sperm quality used in *in vitro* fertilization techniques. Inositol is a polyalcohol, naturally occurring as nine stereoisomers, including D-chiro-inositol and MYO, that belongs to the vitamin B complex group. In male reproductive organs, MYO is mainly produced by Sertoli cells in response to follicle-stimulating hormone (FSH) and is involved in processes that include the regulation of motility, capacitation and acrosome reaction of sperm cells[28,32-34]. Moreover, since concentrations of MYO increase from the epididymis to the deferent duct, this compound is considered important for sperm maturation and for sperm chemiotaxis through production of inositol triphosphate and calcium channels opening[35]. Low concentration of MYO within epididymis has been associated with reduced fertility in transgenic mice[36]. MYO also seems to play a role in osmoregulation in seminal vesicular fluid, contributing to reduce viscosity and increase sperm motility[31]. In relation to this, when added to seminal fluid of patients with oligoasthenoteratospermia, MYO reduces the presence of amorphous material[27]. At a functional level, MYO acts directly on mitochondria increasing the membrane potential and thereby improving sperm motility in patients with altered sperm parameters[37].

In fact, *in vitro* MYO increased the number of spermatozoa with high mitochondrial membrane potential (MMP) and it decreased the number of those with low MMP in OAT patients[37].

Folate is important for the synthesis of DNA, transfer RNA and proteins, and is therefore suggested to be important in spermatogenesis[38]. Although results are controversial[39] and the absence of an effect of folic acid on endocrine parameters[40] (i.e., FSH, testosterone and inhibin B concentrations), some researchers[40,41] demonstrated that folic acid intervention improved sperm concentration and motility in sub-fertile males. Betaine is a natural methylamine that acts as a methyl donor to convert the harmful homocysteine into methionine. Several studies suggested that betaine may have an important role in maintaining male fertility[42,43] and antioxidant proprieties[44,45].

As regarding B vitamins, positive effects of vitamin B12 on sperm count, sperm motility, and reducing sperm DNA damage, may be related to increased energy metabolism or to higher oxygenation due to the boost in red blood cell production[46]. In the present study we aimed at evaluating for the first time the effect of combined treatment with MYO, ALA, folic acid and vitamins on semen parameters of sub-fertile men.

Patients and Methods

Patients

A total of 143 no-smokers sub-fertile men were recruited at UOS Physiopathology of Human Reproduction, Ospedale Policlinico San Martino, Genoa and at Physiopathology of Reproduction and Andrology Unit, Sandro Pertini Hospital, Rome from April 2016 to September 2017. The 143 subjects were analyzed for eligibility: 25 did not meet inclusion criteria and, therefore, were excluded from the study. The remaining 118 subjects were enrolled. Eighteen subjects did not complete the study: 10 were lost at follow-up and 8 for spontaneous pregnancy. Therefore, a total of 100 subjects were included in statistical analysis. The patients were 26-53 years aged (average age: 39.6 $\pm$ 5.9 years), with one or more altered semen parameters according to WHO 2010 criteria[5]. Specifically, at the baseline 16% of patients presented oligospermia, 43% asthenospermia, 41% both oligospermia and asthenospermia. Moreover, patients had a body mass index (BMI) between 20 kg/m^2 and 25 kg/m^2, a normal endocrinological/metabolic profile (homeostatic model assessment (HOMA) index[38] < 2.5; 150 ng/dL < testosterone < 800 ng/dL, 0 ng/ml < prolactin < 20 ng/ml, 1.5 mIU/mL < FSH < 12.4.0 mIU/mL, 6 mIU/mL < LH < 30 mIU/mL serum values), and no concomitant consumption of drugs. Patients with azoospermia or severe oligozoospermia (sperm count less than 5 x 10^6/ml) or with an identifiable cause of infertility (leukocytospermia and/or positive sperm culture, epididymo-orchitis, prostatitis, inguinoscrotal surgery, cryptorchidism, varicocele, etc.) were excluded from the study.

Study Design

Semen analysis was performed at baseline (T0) and after 90 days (T90) of treatment, and results were compared. The study was approved by the Ethical Committee of Regione Liguria (Approval no. 416REG2017) and each patient signed an informed consent to the enrollment in the study.

Treatment Protocol

Diet of patients was supplemented with 2 tablets/day of Sinopol® (Laborest S.r.l., Nerviano, Milan, Italy). Two Sinopol® tablets contained ALA (800 mg), MYO (1000 mg), folic acid (400 mg), betaine (100 mg), vitamin B2 (1.7 mg), B6 (1.9 mg), and B12 (2.6 mg).

Sperm Analysis

At T0 and T90 semen samples were obtained after 3 days of sexual abstinence. They were collected into sterile containers and held at 37°C for 30 minutes to liquefy. After liquefaction, a routine sperm analysis was carried out according to the WHO criteria[5], evaluating sperm number, motility, and morphology. Sperm score for motility evaluation was expressed as grades a to d, and progressive motility rate was calculated as the percentage of a + b.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD). Mann-Whitney test was performed using using MedCalc® software (Mariakerke, Belgium), in order to investigate differences between semen parameters before and after the treatment. Results were considered statistically significant at $p<0.05$.

Results

One hundred and forty-three patients were recruited and began Sinopol® treatment. Out of them, 25 patients did not met inclusion criteria in the stu-

Table I. Semen characteristics of patients before and after treatment.

	Before*	After*	*p*-value
Sperm concentration (x 106/ml)	16.6 + 13.1	24.4 + 23.4	0.0009
Number of spermatozoa (x 106/ml)	46.5 + 38.7	69.1 + 59.0	0.0017
Progressive motility (%)	19.5 + 15.6	24.8 + 16.5	0.0047
Total motile sperm count (x 106)	9.8 + 11.5	22.3 + 30.8	0.0010
Normal sperm morphology (%)	4.9 + 3.1	7.9 + 4.1	< 0.0001

*Data are expressed as mean + SD

dy mainly due to history of genital diseases as epididymo-orchitis, prostatitis, cryptorchidism, current genital inflammation or varicocele that came to light after Sinopol® prescription. Among the 118 men that fulfilled inclusion criteria, 10 (8.4%) patients were lost at follow-up and in 8 (6.8%) cases the partner got pregnant spontaneously. A total of 100 patients completed the whole study (Figure 1). The patients were 28-52 years aged (average age: 37.7 + 4.7 years). No side effects due to the oral administration of Sinopol® were observed in any participants. Sperm parameters of the 100 participants at baseline and at the end of the study are presented in Table I. Analyses of data showed a statistically significant increase of sperm concentration (percentage increase=+41.2%, p=0.0009), of number of spermatozoa (percentage increase=+50%, p=0.0017), of progressive motility (percentage increase=+31.6%, p=0.0047), of total motile sperm count (percentage increase=+120%, p=0.0010), and of normal sperm morphology (percentage increase=+60%, p<0.0001) after the treatment respect on the baseline. We also registered the reproductive follow-up of the 100 patients that completed the study. Within

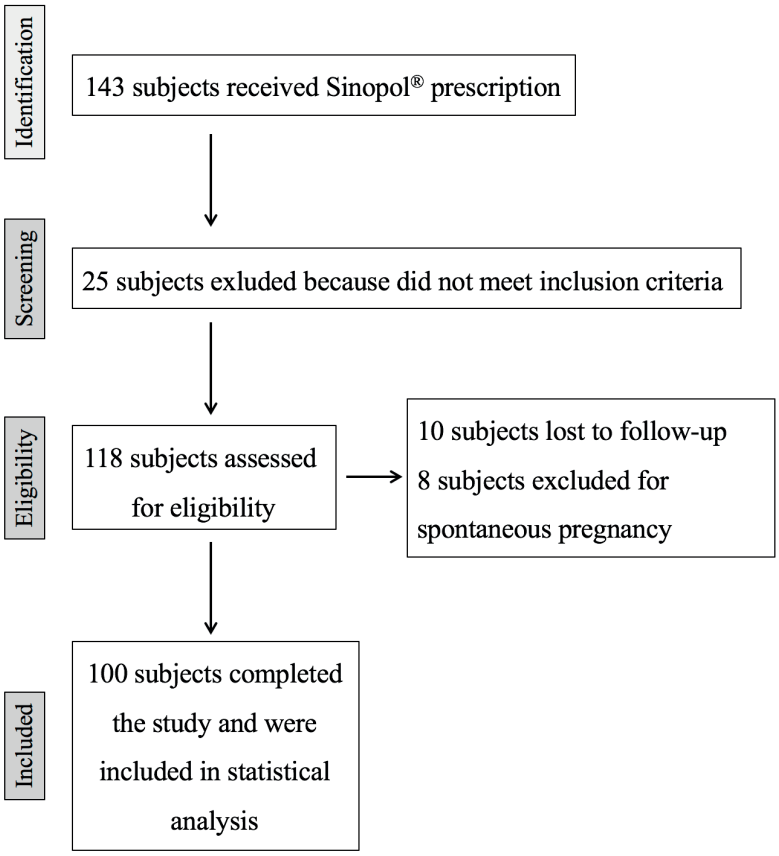

Figure 1. Flowchart of patient recruitment for the study of Sinopol® supplementation in sub-fertile men.

6 months following Sinopol® discontinuation, the partner got pregnant in 40 cases (6 spontaneously, 4 with intra-uterine insemination, and 30 after ICSI or FIVET treatment).

Discussion

This multi-centric study represents the first evaluation of effects of combined treatment with myo-inositol, alpha-lipoic acid, folic acid, betaine and vitamins of sub-fertile men. After 3 months of co-administration of these dietary supplements, a significant improvement of sperm parameters was observed. Regulation of sperm mobility is largely controlled at the mid-piece: the sperm flagellum handles the activation of motility, and the principal mid-piece controls hyperactivation[48]. The mid-piece is rich in unsaturated lipids and saturated protein channels that are vulnerable to free radical attack. Therefore, the anti-oxidant properties of ALA and MYO may protect these components and, in turn, ensure their structural and functional integrity[49]. The rate of sperm motility is also highly dependent on its energy supply and thus on external and internal structural integrity of mitochondria[50]. At the same time, very active mitochondria of normal function sperms create high levels of free radicals as by-products of the Krebs cycle. Therefore, antioxidant addition would protect these organelles from free radicals attack and ensure a constant yield of ATP[21]. The ALA is thought to assist the metabolism of oxidative decarboxylation by acting as a co-enzyme, thus increasing cytochrome C concentration and directly raising the mitochondria's membrane potential, likewise MYO does[27,35,51]. Moreover, MYO has been suggested to play a role in sperm chemotaxis through the activation of phospholipase C, resulting in the production of inositol triphosphate and calcium channels opening[52], and through reduction of the semen amorphous material. Therefore, the synergic effect of ALA and MYO would result in a positive effect on sperm mobility, as observed.

We also found that administration of ALA and MYO was able to increase sperm number. This finding may be attributed to the normalized oxidative stress which on one hand may modulate proliferation of germ cell, protected against DNA fragmentation and membrane damages, and on the other may maintain signal transduction mechanisms necessary for normal function of hypothalamus-testicular axis. On the whole, the defensive influence of ALA and MYO against oxidative reproductive damage may lead to normal secretion of testosterone and sperm production. With regards to this issue, it has been demonstrated that ALA sustained sex-hormone-binding globulin and dehydroepiandrosterone sulfate serum concentration, the levels of testicular cholesterol, and glucose-6-phosphatedehydrogenase and 3β-hydroxysteroid dehydrogenase activities in testis[23]. Moreover, with oral administration, MYO has been indicated to improve the hormonal milieu of the testis. Specifically, MYO is able to rebalance serum luteinizing hormone, follicle-stimulating hormone (FSH), and inhibin B concentrations[28]. Since FSH plays a key role in control of Sertoli cell number and function, that are vital for normal spermatogenesis, it is possible that MYO may enhance the function of these nurse cells. MYO seems to play an important role in the maturation of sperms also during the migration from the epididymis. In fact, MYO shows a gradient of concentration, most abundant in the seminal plasma increasing along epididymis and deferent ducts[53]. In addition, folic acid and vitamin B12 contained in Sinopol® may contribute to the improvement of semen characteristics found at the treatment discontinuation. Folate may be beneficial for spermatogenesis since it is required for the *de novo* synthesis of purines, thymidylate and methionine[38]. Although the underlying mechanism involved is not clarified and results are controversial[39], some authors demonstrated that folic acid improved number and motility of sperm[40,41]. A recent review demonstrated the positive impact of vitamin B12 on semen quality by increasing sperm count and by enhancing sperm motility and reducing sperm DNA damage[54]. The beneficial effects of vitamin B12 on semen quality may be due to increased functionality of reproductive organs, decreased homocysteine toxicity, reduced amounts of generated nitric oxide, decreased levels of oxidative damage to sperm, reduced amount of energy produced by spermatozoa, decreased inflammation-induced semen impairment, and control of nuclear factor-kB activation[54]. The strength of this study is that it showed for the first time that the co-treatment with MYO and ALA, combined with folic acid and vitamins, can improve semen quality of dispermic men. In fact, up to now it has been demonstrated the positive impact on infertile men of the individual administration of MYO or ALA[24,28,30,55], and at present Sinopol® is labeled as a dietary supplement to counteract nutritional deficiencies in women affected by PCOS (www.

laborest.com/en/products/sinopol/sinopol-fast-slow-tablets). Moreover, we not only reported no adverse effects of this combined treatment on the male subjects, but we also demonstrated that a lower dosage of MYO and ALA than those previously found to be tolerated by human subjects[56] or used in infertile men[24,28,30,56] is properly effective, probably due to a synergic effect between ALA and MYO.

Conclusions

For the first time we reported that a combination of nutraceuticals composed of myo-inositol, alpha-lipoic acid, folic acid, betaine and vitamins improves sperm parameters in sub-fertile men. We are aware that to clarify the clinical relevance of the data more investigations with larger sample sizes and longer durations are needed, as well as evaluation of MYO and ALA co-treatment effectiveness in improving the chances to obtain a pregnancy spontaneously or following assisted reproduction.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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