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Correspondence:
Aldo E. Calogero, Section of Endocrinology, Andrology and Internal Medicine, Department of Clinical and Experimental Medicine, University of Catania, Policlinico "G. Rodolico", Via S. Sofia 78, Building 4, 95123 Catania, Italy.
E-mail: acaloger@unict.it

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Myoinositol improves sperm parameters and serum reproductive hormones in patients with idiopathic infertility: a prospective double-blind randomized placebo-controlled study

[1]A. E. Calogero, [2]G. Gullo, [1]S. La Vignera, [1]R. A. Condorelli and [3]A. Vaiarelli

[1]Section of Endocrinology, Andrology and Internal Medicine, Department of Clinical and Experimental Medicine, University of Catania, Catania, [2]Dipartimento di Ostetricia Ginecologia e Medicina Interna, Università degli Studi di Messina, and [3]Reproductive Medicine Unit, Policlinico Universitario di Messina, Messina, Italy

SUMMARY

Male infertility is a multifactorial disorder that affects a significant percentage of couples. Its etiology and pathogenesis remain elusive in about one-third of the cases; this is referred to as idiopathic infertility. Inositols mediate the sperm processes involved into oocyte fertilization, such as penetration of the ovum cumulus oophorus, binding with the zona pellucida and the acrosome reaction. The aim of this double-blind, randomized, placebo-controlled trial was to evaluate the efficacy and safety of myoinositol (the most abundant form of inositols present in nature) treatment in men with idiopathic infertility. To accomplish this, we evaluated the effects of myoinositol on sperm parameters and reproductive hormones at baseline and after 3 months of treatment in men with idiopathic infertility. No adverse reaction was observed. Myoinositol significantly increased the percentage of acrosome-reacted spermatozoa, sperm concentration, and total count and progressive motility compared to placebo. In addition, myoinositol rebalanced serum luteinizing hormone, follicle-stimulating hormone, and inhibin B concentrations. The clinical improvement of idiopathic infertile patients should encourage myoinositol use for the treatment of this disorder, even though its detailed mechanisms at the testicular level remain still unclear.

INTRODUCTION

Infertility is a worldwide problem which strongly impacts both medically and psychosocially in couples in reproductive age (Fisher & Hammarberg, 2012). Male partner contributes to about 40% of cases of infertility (Alam, 2009). Conditions such as varicocele, cryptorchidism, and hypogonadism are among the many causes of male infertility. Nevertheless, a significant proportion of male infertility undergoes undiagnosed despite an extensive diagnostic workout. This is referred to as idiopathic infertility (Siddiq & Sigman, 2002; Deng *et al.*, 2008) which is characterized by sperm parameters below the World Health Organization (WHO) reference values (WHO, 2010).

Spermatozoa acquire motility during the epididymal transit, becoming able to migrate from the vagina to the Fallopian tubes, to penetrate the cumulus oophorus and to carry out all those processes involved in fertilization (Beauchamp *et al.*, 1984). At first, after penetrating the cumulus oophorus of the ovum, the spermatozoon binds to the zona pellucida with its plasma membrane intact; then it undergoes to the acrosome reaction (Kopf & Gerton, 1991). This results in the release of hydrolytic enzymes that digest the zona pellucida, allowing to the spermatozoon to penetrate into the oocyte and to fertilize it (Breitbart & Spungin, 1997).

All these events are allowed by inositols and particularly by increasing intracellular Ca^{2+} release through inositol-gated channels because of the protein kinase PKC activation (Roldan & Shi, 2007). Among inositols, the most abundant form in nature is myo-inositol (MI), a member of vitamin B complex group, involved in several systemic processes and mechanisms of signal transduction in the plasma membrane as precursor of second messengers (Condorelli *et al.*, 2011). Interestingly, MI is involved in the regulation of intracellular Ca^{2+} concentration (Foskett,

2010) and previous evidence suggests a possible role of MI in spermatogenesis and sperm function. Indeed, in transgenic mice, low MI concentration within epididymis has been associated with reduced fertility (Yeung *et al.*, 2004). Moreover, MI increased the number of spermatozoa with high mitochondrial membrane potential (MMP) while it decreased the number of those with low MMP in patients with OAT in vitro (Ching-Hei 2004; Foskett, 2010; Condorelli *et al.*, 2011; Condorelli *et al.*, 2012).

On this basis, we conducted a double-blind, randomized, placebo-controlled trial to evaluate the efficacy and safety of MI (Inofolic; Lo.Li Pharma s.r.l., Rome, Italy), in men with idiopathic infertility. To accomplish this, we examined the effects of MI on sperm parameters and serum reproductive hormones at baseline and after 3 months of treatment with MI or placebo.

MATERIALS AND METHODS

Study population and inclusion/exclusion criteria

A total of 194 patients with idiopathic infertility who did not achieve a pregnancy after more than 2 years of unprotected intercourse with the same fertile partner were enrolled in this study. The patients included were younger than 45 years, with an average age of 28 ± 9 years for the group that received MI and 28 ± 10 years for the group that received placebo and a body mass index (BMI) of 26.6 ± 2.7 years for the group that received MI and 26.8 ± 2.4 years for the group that received placebo. Patients with azoospermia or severe oligozoospermia (sperm count less than 5 million/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.

Randomization and treatment protocol

A 3-month, randomized, placebo-controlled double-blind study was performed to compare the effects of MI against placebo. Eligible participants were randomly allocated to one of two groups. Group 1 (n = 98) received Inofolic (Lo.Li Pharma s.r.l.) as one sachet twice daily. Group 2 (n = 96) received one identical placebo sachet twice daily.

Inofolic sachet contained 2 g of MI and 200 μg of folic acid. Placebo sachets contained folic acid alone. All patients and study personnel were unaware of group treatment assignment. The duration of the treatment was arbitrary chosen according to the physiological length of spermatogenesis.

Serum hormone measurement and sperm analysis

All patients were evaluated with a complete medical and reproductive history, detailed physical examination and sperm analysis. The hormonal evaluation, by radioimmunoassay, included assays for luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), inhibin B and testosterone. Blood sampling was performed at 8 AM, after at least 8 h of sleep. Serum LH and PRL measurements were repeated twice after a 30-min interval. Hormonal examinations were performed on the day before the treatment was begun and after its discontinuation.

The percentage of sperm acrosome reaction was evaluated using a previously published methodological protocol (Chiu *et al.*, 2005).

The semen samples were obtained after 3 days abstinence. They were collected into sterile containers and allowed to liquefy at 37 °C for 30 min. A routine sperm analysis was carried out according to WHO criteria (WHO, 2010). Two semen samples were analyzed at baseline, separated by 5–7 days, and immediately after therapy discontinuation.

The study was approved by the Internal Institutional Board and an informed signed consent was obtained from all patients.

Statistical analysis

Data were expressed as mean ± SD and statistically analyzed by analysis of variance (ANOVA) followed by the Bonferroni post-test. Differences in the percentage of acrosome-reacted spermatozoa were evaluated by Fishers exact test. Statistical analysis was performed by using GraphPad Instat software (GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant at the level of p lower than 0.05.

RESULTS

A total of 194 patients were randomized at baseline to receive MI (4 g/day, n = 98) or placebo (n = 96) for 3 months. The two groups did not differ significantly for age, BMI, serum hormone concentrations, sperm parameters, and percentage of acrosome-reacted spermatozoa (Table 1).

Table 1 BMI, hormonal values and sperm parameters at baseline in patients treated with myoinositol or placebo

Parameters	Myoinositol (n = 98)		Placebo (n = 96)	
	Before treatment	After treatment	Before treatment	After treatment
BMI (kg/m^2)	26.6 ± 2.7	25.9 ± 2.5	26.8 ± 2.4	26.4 ± 2.2
LH (IU/L)	12.1 ± 2.6	8.8 ± 2.6*	12.4 ± 2.4	12.6 ± 2.4
FSH (IU/L)	16.7 ± 4.1	10.7 ± 4.1*	16.6 ± 4.1	16.8 ± 4.2
Prolactin (pmol/L)	374 ± 120	368 ± 118	361 ± 123	365 ± 121
Inhibin (ng/L)	86.0 ± 24.0	105.0 ± 28.0*	87.0 ± 25.0	88.0 ± 25.0
Testosterone (nmol/L)	15.8 ± 5.4	18.6 ± 5.6	15.6 ± 4.8	15.8 ± 4.6
Ejaculate volume (mL)	2.7 ± 1.3	2.7 ± 1.4	2.8 ± 1.4	2.7 ± 1.7
Sperm concentration (million/mL)	20.2 ± 4.6	26.4 ± 4.4*	20.4 ± 4.4	20.8 ± 4.3
Total sperm count (spermatozoa/ejaculate)	46.6 ± 12.6	57.6 ± 14.4*	47.2 ± 12.2	47.8 ± 11.2
Progressive motility (%)	22.2 ± 2.1	27.6 ± 1.8*	22.3 ± 2.6	23.3 ± 2.1
Acrosome-reacted spermatozoa (%)	34 ± 8	41 ± 11*	35 ± 8	36 ± 10

BMI, Body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone. *p < 0.05 vs. after treatment with placebo.

Treatment with MI or placebo did not affect serum PRL and testosterone concentrations as well as ejaculate volume compared to baseline values. On the other hand, MI significantly increased serum inhibin B concentration, sperm concentration, total sperm count, the percentage of spermatozoa with progressive motility and acrosome-reacted spermatozoa ($p < 0.05$) compared to placebo. Moreover, MI significantly reduced FSH and LH concentration ($p < 0.05$) vs. placebo.

Finally the most common side effects reported in literature (nausea, flatus, and diarrhea) were observed in some patients during the study. None of them required the discontinuation of the treatment. This may be ascribed to the dose used, that was lower than that reported associated with side effects (12 g/day) (Carlomagno & Unfer, 2011).

DISCUSSION

This double-blind, placebo-controlled randomized study represents the first evaluation of MI effects for the treatment of patients with idiopathic infertility. After 3 months of treatment, MI was able to improve hormonal and sperm parameters in these patients compared to the effect observed following administration of placebo.

Interestingly, MI was able to slightly but significantly decrease serum FSH levels, notoriously increased in infertile patients with primary testiculopathy (Shulman *et al.*, 1999). It is well-known that FSH plays a key role in the control of Sertoli cell number and function (Orth, 1982), promoting the differentiation of these cells that are essential to sustain a normal spermatogenesis (Griswold, 1993). Evidences suggest that MI, acting as second messenger, regulates the activities of hormones such as FSH, becoming useful in contrasting the syndromes in which this hormone is quantitatively altered, such as polycystic ovary syndrome (Iuorno *et al.*, 2002; Papaleo *et al.*, 2007, 2009; Dinicola *et al.*, 2014). In the seminiferous tubules, the concentration of MI is higher than that found in the seminal plasma, even though its true role remains still poorly understood. Furthermore, the precise molecular and biochemical mechanisms involved in FSH regulation of Sertoli cell function remain largely unknown.

It is tempting to speculate that, in male patients, MI could modulate the PKA-, PKB-, and PKC-dependent pathways that influence Sertoli cell response to FSH (Lambert *et al.*, 1991; Meroni *et al.*, 1998; Meroni *et al.*, 2002), mobilizing Ca^{2+} (Nishizuka, 1992; Newton, 1995). Indeed, free extracellular Ca^{2+} is mandatory for sperm motility and acrosome reaction (Yanagimachi & Usui, 1974; Florman *et al.*, 1989), even though evidence reported some cases of acrosome reaction in a calcium-independent manner (Rotem *et al.*, 1992). In particular, two possible roles for PKC in acrosome reaction have been proposed: the first is to trigger a plasma membrane calcium channel to increase intracellular Ca^{2+} (Galizzi *et al.*, 1987; Spungin & Breitbart, 1996); the second one is to activate the phospholipase A_2 (PLA_2) that generates arachidonic acid (Roldan *et al.*, 1992). Interestingly, a crosstalk between PKA and PKC during sperm capacitation was highlighted by Cohen and colleagues (Cohen *et al.*, 2004; Breitbart *et al.*, 2005) with a consequent rapid increase in actin polymerization which is followed by fast depolymerization, probably because of the increase in Ca^{2+} levels. Therefore, spermatozoa ability to fertilize is tightly associated to a remodeling in cytoskeletal compounds. It is well-known that inositols are involved in cytoskeletal reorganization and in cellular migration (Le Clainche & Carlier, 2008; Windhorst *et al.*, 2008; Johnson & Schell, 2009), and it is likely that MI could ameliorate the fertility rate of the patients with idiopathic infertility acting both on spermatozoa cytoskeleton rearrangements and on their migratory ability.

In addition to the effects on serum FSH levels, MI administration lowered LH concentration. These gonadotropins are strictly associated each other and high concentrations of FSH and LH in serum were found in case of low sperm concentration (de Kretser, 1979; Abramsson & Duchek, 1989). On the other hand, MI increased the levels of Inhibin B, a glycoprotein secreted from the testis as a product of Sertoli cells involved in the regulation of FSH secretion by a negative feedback mechanism (Illingworth *et al.*, 1996; Hu & Huang, 2002). In men with normal and altered spermatogenesis a strong inverse correlation has been reported between inhibin B and FSH levels (Anawalt *et al.*, 1996; Klingmüller & Haidl, 1997). Moreover, inhibin B concentrations are closely related to sperm concentration in the ejaculate (Jensen *et al.*, 1997) and to testicular volume (Pierik *et al.*, 1998). It has been suggested that inhibin B may be a better predictor of spermatogenesis than FSH (Jensen *et al.*, 1997; Pierik *et al.*, 1998). Indeed, diagnostic accuracy of FSH is limited by the fact that spermatogenic arrest at late stages does not lead to changes in FSH secretion and that FSH may be normal in patients with total absence of germ cells or hypospermatogenesis (Bergmann *et al.*, 1994).

The acrosome reaction is a prerequisite for fertilization in mammals. It consists of plasma membrane and the outer acrosome membrane fusion above the anterior portion of the sperm head. It takes place on the surface of the zona pellucida, after specific binding with a specific glycoprotein, the ZP3 (Wassarman & Litscher, 1995). When the acrosome reaction has been completed, the spermatozoon is able to penetrate the zona pellucida. The acrosome reaction is evaluated over time to evaluate its real potential. In fact, a premature acrosome reaction leads to a loss of the recognition sites of the zona pellucida on the surface of the spermatozoon and thus may affect gamete fusion (Tesarik, 1989). In contrast, sperm activation inability, responsible for initiation of the acrosome reaction, prevents the oocyte penetration. The measurement of spontaneous acrosome reaction in the ejaculate does not offer any information about this function. It can be concluded, therefore, that the study of spontaneous acrosome reaction is not a true test of function, but represents a dysfunctional test. It occurs, for example, in patients who show in a short time a high proportion of acrosome reaction, as a result of acrosome instability. Thus, further studies are needed to investigate this important functional aspect.

In conclusion, the results of this study showed that MI is a safe supplement able to significantly rebalance serum gonadotropin and inhibin B levels and to increase sperm parameters in patients with idiopathic infertility. MI contributed to balance the hormonal and sperm biomarkers, as it acts as second messenger regulating the activities of several hormones (Bizzarri & Carlomagno, 2014). Hence, patients with idiopathic infertility take advantage by MI supplementation which should be encouraged in these patients.

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