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Myo-inositol impact on sperm motility in vagina and evaluation of its effects on foetal development

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Abstract. – OBJECTIVE: To counteract the arising problem of couple infertility, having good quality gametes is increasingly important. A molecule that seems to be useful to favor this condition is myo-inositol (MI), the most common stereoisomer of the inositol family, involved as second messenger in several cell pathways (osmoregulation, chromatin remodeling, gene expression, etc.). To evaluate this possibility, a treatment with myo-inositol in idiopathic infertile couples was performed in this randomized, placebo-controlled study.

PATIENTS AND METHODS: 86 couples were enrolled and randomly assigned to two groups, treated either with MI (Xyminal®, Lo.Li. Pharma Srl, Rome, Italy) or placebo suppositories, to evaluate the effects on sperm motility, cervical mucus quality and pregnancy rate. Moreover, in pregnancy cases, all routine controls on gestation progress and foetal health were performed to confirm the safety of this treatment.

RESULTS: As showed in this study, MI treatment allows an increase of total sperm motility (54.42 ± 8.72) in comparison to placebo group (46.21 ± 5.33). Moreover, MI mildly improves cervical mucus quality and increases the number of pregnancies (18.60%) in comparison to the placebo group (6.97%).

CONCLUSIONS: MI improves sperm motility and cervical mucus quality, increasing the probability of conception. The absence of adverse events both for the mother and the foetus confirmed the safety of this molecule in pregnancy, supporting even more its use for couples seeking pregnancy.

Key Words:
Myo-inositol, Idiopathic infertility, Sperm motility, Foetal development, Cervical mucus.

Introduction

Nowadays, the concept of infertility, defined as the impossibility to achieve pregnancy during 12 months of focused attempts, is a condition involving about 100 million people in the world. In this regard, new statistics data have confirmed that the male factor is responsible of 20-50% of couple infertility cases[1]. Although male fertility can be affected by a great number of pathologies as cryptorchidism, varicocele or genital infections, there are many male patients without a full-blown aetiological factor able to explain their infertility; in these cases, the infertility is defined *idiopathic*. Frequently, this condition is characterized by increased production of Reactive Oxygen Species (ROS), which causes the depletion of sperm antioxidants[2]. Physiologically, spermatozoa have a low antioxidant capacity and, for this reason, they can be easily affected by ROS. To help sperm functions, particularly in the case of idiopathic infertility[3], a constant supplementation with antioxidants, starting from sperm cell development to conception, is fundamental. These molecules are free-radical scavengers and protect spermatozoa by counteracting lipid membrane peroxidation, protein corruption and DNA fragmentation[4]. In this regard, the importance of inositols as antioxidants is well documented[5-12]. Inositols are polyols with a six-carbon cyclic structure, with a hydroxyl group on each carbon atom. Myo-inositol (MI) is the most common stereoisomer[13]. MI constitutes an important element of membrane phospholipids, mediates osmoregulation[14] and mediates protein phosphorylation[15], participates in chromatin remodeling and gene expression[16,17], and facilitates mRNA export[18]. Phosphorylated MI derivatives, instead, act as second messengers in several pathways[19]. In sperm cells, MI is involved in many transduction mechanisms responsible for cytoplasmic calcium level regulation, capacitation, mitochondrial functionality and involved in the frequency of tail beating. The presences of MI in the seminal fluids and of enzymes, responsible of its synthesis (myoinositol-1-phosphate synthase, ISYNA1 and myoinositol-monophosphatase-1,

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IMPA1) in the testicles, allows us to hypothesize a role for this molecule in sperm maturation[9,20,21]. In this regard, an increased compensatory production of the enzymes responsible of MI synthesis, in asthenozoospermic patients, was reported in several studies[22]. A further confirmation of MI role in male fertility derives from some *in-vitro* studies that demonstrated a significant increase in spermatozoa motility from oligoasthenoteratozoospermic men, when incubated with MI[5,8]. Finally, there are several *in-vitro* fertilization (IVF) studies, which report improved sperm cell motility and increased percentage of fertilization in intracytoplasmic sperm injection (ICSI) procedures, after incubation with MI[5,23]. The importance of MI for couple fertility is not limited on male factor but also affects the female fertility. Indeed, in addition to its well-known activity on sperm cells, in particular on spermatozoa motility[5,8], there are several studies on its effects on cervical mucus[24], on oocyte quality[25-29] and on the restoration of a regular menstrual cycle in women with PCOS[30-35]. Moreover, the importance of myo-inositol for newborn health is reported by Greene et al[36], which investigate its ability to potentiate the protective activity of folic acid against the development of neural tube defects. For all these reasons, the aim of this randomized, placebo-controlled study is to evaluate the effect of myo-inositol on sperm functionality, cervical mucus quality and pregnancy rate in idiopathic infertile couples, and to report the possible impact on the offspring. As the primary outcome of this study, we wanted to investigate whether MI vaginal suppositories, given to fertile females, can ameliorate the impaired motility and poor quality of their partners' sperm. The secondary outcomes were the improvement of cervical mucus quality, the increase of pregnancy rate and the assessment of MI vaginal suppositories safety for pregnancy and offspring.

Patients and Methods

Target Population and Inclusion/ Exclusion Criteria

Between January 2017 and September 2019, 86 idiopathic infertile couples, aged 25-39 years, under treatment at the of Reproductive Medicine Centre of Altamedica Unit (Rome, Italy) for failing to achieve pregnancy after 12 months of unprotected intercourse, were enrolled in this study. Inclusion criteria were females characterized by a regular menstrual cycle, normal hormonal pattern and patent tubes and males with sperm within the range between the 5th and the 25th percentile for total motility, according to the parameters of the World Health Organization on male fertility evaluation (WHO). Any other endocrine, metabolic, autoimmune, neoplastic diseases in both female and male partners will be considered as exclusion criteria. All subjects involved provided written Informed Consent Form before participation, according to the Declaration of Helsinki. The study protocol was evaluated by the local Ethics Committee.

Protocol Design and Treatment

The included couples were randomly assigned to two groups: 43 couples were treated with MI vaginal suppositories (Xyminal®, Lo.Li. Pharma Srl, Rome, Italy) while the remaining 43 couples were treated with placebo suppositories. Both groups underwent 1-3 consecutive cycles with vaginal suppositories until pregnancy. Each cycle consisted in using 3 suppositories, one every other day, in the periovulatory time, inserted in vagina before bedtime. Specifically, if the ovulation day was the 14th day of the menstrual cycle, one suppository had to be taken on the 11th, one on the 13th and a final one on the 15th day of the menstrual cycle. To define precisely menstrual cycle phases, follicle diameters were evaluated by ultrasound measurement. When the leading follicle was >16 mm, the female partners started the treatment with MI vaginal suppositories. Before the first cycle of treatment, a baseline spermiogram analysis was performed between the 7th and the 9th day of the menstrual cycle, according to WHO references[37]. During the periovulatory phase, the couples were asked to have a sexual intercourse the day after the last suppository application of each cycle (with follicle >18 mm), possibly in the morning. Finally, a postcoital sperm test on vaginal smear was performed 3-6 hours after the intercourse. If the conception was achieved, the couples were monitored for the entire pregnancy duration, until 15 days after childbirth. All data about the pregnancy, such as ultrasounds at 12, 21, 32 weeks, APGAR indexes, and neonatological evaluations were recorded.

Spermiogram Analysis

All male participants were subjected to a spermiogram analysis for baseline sperm parameters evaluation. Semen samples were obtained after 3 days of sexual abstinence. All samples were collected into sterile containers, stored at 37°C,

allowed to liquefy for 30 min and subsequently assessed according to World Health Organization Laboratory Manual[37]. The parameters evaluated were semen volume, semen pH, total sperm number, sperm concentration, viscosity, fluidification, density, total motility, progressive motility, not progressive motility, static spermatozoa morphology and vitality.

Postcoital Test (PCT)

PCT was performed 3-6 hours after coitus, as described by Hull et al[38] and according to the WHO procedure[37]. The exocervical mucus was removed with a swab and the cervical mucus was collected from the endocervical canal by aspiration with a tuberculin syringe (without needle). The evaluation of cervical mucus properties included the assessment of spinnbarkeit, ferning (crystallization), viscosity, and pH, according to the system devised by Moghissi[39]. Sperm total and progressive and non-progressive motility were evaluated following WHO criteria[37].

Statistical Analysis

Sample size calculation was performed by nQuery Advisor 7.0 software (Statistical Solutions, Saugus, MA, USA), based on Chi square test, to obtain 80% power with a maximum drop-out of 10% at an alpha risk of 5% assuming a pregnancy rate of 80% for couple without apparent troubles of fertility and supposing 25% improvement of pregnancy rate in treated group. Results are presented as mean ± standard deviation (SD). Differences were analyzed with Student's paired *t*-test. $p < 0.05$ was considered statistically significant.

Results

In this study, 86 couples were enrolled. All patients completed correctly the study. Patients' average baseline characteristics are summarized in Table I. As reported in Table II, MI treatment was able to improve the overall sperm motility at postcoital test. Indeed, due to its physical-chemical characteristics, MI allowed to increase the total sperm motility (54.42 ± 8.72) when compared either to baseline (46.48 ± 4.05) and to placebo group (46.21 ± 5.33). Of note, as clearly reported, the treatment increased the portion of spermatozoa with progressive motility (49.68 ± 6.74) when compared either to baseline (39.33 ± 4.60) and to placebo group (38.46 ± 4.73), an essential result when pregnancy is the intercourse aim. In this regard, it is important to underline that progressive motility is considered the best marker to identify good quality and healthy sperm cells. Hence, this type of spermatozoa, if available, is normally used for IVF techniques. In addition to this effect, MI allowed a mild improvement of cervical mucus quality, reducing viscosity, spinnbarkeit and ferning. The MI effect on fertility can also be confirmed by evaluating the pregnancy rate of each group. Indeed, thanks to this treatment, a greater number of couples (18.60%) were able to

Table I. Real time-PCR primer sequence.

Characteristics	Females	Males
Age	34.63 ± 3.81	35.76 ± 3.04
Weight (kg)	58.18 ± 5.36	79.32 ± 4.15
Height (cm)	168.52 ± 8.71	178.37 ± 10.31
BMI	22.71 ± 2.21	25.61 ± 1.843
Total motility* (PR + NP) (%)	–	46.48 ± 4.05
PR*	–	39.33 ± 4.60
NP*	–	7.15 ± 1.24
IM*	–	53.52 ± 8.12
Semen volume* (mL)	–	3.17 ± 0.81
Total sperm number* (106 per ejaculate)	–	70.46 ± 27.42
Sperm concentration* (106 per mL)	–	24.28 ± 8.88
Sperm morphology* (normal forms, %)	–	10.71 ± 4.09
Viscosity	2.85 ± 0.37	-
Spinnbarkeit	8.54 ± 1.22	-
Ferning	2.88 ± 0.71	-
pH	7.62 ± 0.51	-

BMI = Body Mass Index; PR = Progressive Motility; NP = Non-progressive Motility; IM = Immotile sperm cells. Data reported as Average ± Standard Deviation. *Parameters obtained by spermiogram.

Table II. Postcoital test results.

Parameters	Treated	Placebo
Total motility (PR + NP) (%)	54.42 ± 8.72*,**	46.21 ± 5.33
PR	49.68 ± 6.74*,**	38.46 ± 4.73
NP	4.74 ± 2.01*,**	7.75 ± 1.91
IM	45.58 ± 4.66*,**	53.79 ± 4.22
Sperm Number per HPF	3.34 ± 1.43	3.23 ± 1.53
Viscosity	2.71 ± 0.56*,**	2.88 ± 0.45
Spinnbarkeit	8.41 ± 0.73**	8.58 ± 0.91
Ferning	2.51 ± 0.63*,**	2.69 ± 0.73
pH	7.37 ± 0.32	7.47 ± 0.49

PR = Progressive Motility; NP = Non-progressive Motility; IM = Immotile sperm cells; HPF = High-Power Field. Data reported as Average ± Standard Deviation; **vs.* control ($p<0.05$), ***vs.* placebo ($p<0.05$)

reach a pregnancy, if compared with the placebo group (6.97%), further strengthening the positive effect of this molecule on human fertility. To exclude any potential negative effect of MI vaginal suppositories on the offspring, the pregnancies achieved during this study were monitored until childbirth. All pregnant women were subjected to routine controls in accordance with their physiological conditions. Three ultrasound investigations, respectively at 12, 22 and 32 weeks, were performed on all women without revealing any anomaly either for the foetuses, the mothers or the placental development. These data confirm the safety of MI for use in pregnancy. Moreover, as reported in Table III, MI treatment resulted totally safe for newborns as showed by APGAR score. Finally, all newborns were subjected to the neonatological evaluation 7-10 days after childbirth, and no adverse events connected with the treatment were reported.

Discussion

Changes around us, more and more influence our decisions, prompting us to prioritize the work at the expenses of the private life. For this reason, an increasing number of couples seeks pregnancy around 35-40 years, a period characterized by a strong fertility reduction. The main consequences are decreased birth rates and increased infertility worldwide. Considering this, infertility definition and diagnostic sperm analysis were recently reformed, lowering the threshold values based on statistics data related to the world population. Good quality gametes are fundamental to increase

Table III. Pregnancy data.

Parameters	Treated	Placebo
Number of pregnancy (M-F)	8 (4-4)	3 (1-2)
Pregnancy rate* (%)	18.60	6.97
Preterm labor** (% – no. of cases)	0.00 – 0	33.33 – 1
Gestational diabetes** (% – no. of cases)	0.00 – 0	0.00 – 0
High blood pressure** (% – no. of cases)	0.00 – 0	0.00 – 0
Infections** (% – no. of cases)	0.00 – 0	0.00 – 0
Preeclampsia** (% – no. of cases)	0.00 – 0	0.00 – 0
Miscarriage** (% – no. of cases)	0.00 – 0	0.00 – 0
Still birth** (% – no. of cases)	0.00 – 0	0.00 – 0
Average APGAR Score§	9.87 ± 0.35	9.67 ± 0.58
APGAR heart rate§	2.00 ± 0.00	2.00 ± 0.00
APGAR respiration§	2.00 ± 0.00	2.00 ± 0.00
APGAR muscle tone§	2.00 ± 0.00	2.00 ± 0.00
APGAR skin colour§	1.87 ± 0.35	1.66 ± 0.58
APGAR reflex irritability§	2.00 ± 0.00	2.00 ± 0.00

M = Number of Males; F = Number of Female. *Percentage calculated compared Data obtained to Total Couples of Group. **Percentage calculated compared Data obtained to Number of Pregnancy. §Data reported as Average ± Standard Deviation

the chances of conception and the identification of safe substances able to increase sperm motility, or favor physiological vaginal conditions, increases the chances of a normal foetus development. Notably, as clearly reported in this paper, MI can reach all these goals. The vaginal application of MI allows to improve the biological conditions for achieving conception, as confirmed by the increased number of pregnancies reported in the treated group. In this regard, improvements of spermatozoa motility and cervical mucus quality were observed without any adverse event.

As reported in literature, MI have confirmed in this study its effect on sperm motility. The innovative data shown in this paper is the ability of this molecule to improve also the conditions of vaginal environment, favoring conception achievement, an effect that was resulted totally safe both for the couples and for the newborns. For all these reasons, although more studies are needed to confirm all data on a wide study population, we recommend MI vaginal application to increase chances of conception.

Conclusions

Data obtained in this study allow to support MI treatment in couple with fertility issues, being useful to achieve pregnancy and totally safe both for the mother and the newborn. This effect could be explained considering the physical-chemical characteristics of this molecule. Thanks to its antioxidant properties[5-12], MI improves sperm motility, by reducing the portion of spermatozoa with an irregular or absent motility, and cervical mucus quality, favouring a better viscosity, two fundamental requirements to achieve pregnancy. Further studies are needed to better clarify *in vivo* the crosstalk between spermatozoa and cervical mucus, in order to understand the mechanisms that MI might trigger and control to improve fertility outcome.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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