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Myo-inositol rather than D-*chiro*-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial

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Abstract. – *Objective:* **Polycystic ovary syndrome (PCOS) is the most common cause of infertility due to menstrual dysfunction, and the most promising treatments for this disease are insulin sensitising agents. *Myo*-inositol and D-*chiro*-inositol are insulin sensitizing agents used in PCOS treatment.**

In the present paper, we aimed to compare the effects *myo*-inositol and D-*chiro*-inositol on oocyte quality in euglycemic PCOS patients.

Materials and Methods: **Eighty-four euglycemic PCOS patients, undergoing ovulation induction for ICSI, were recruited for this study. Forty-three participants received *Myo*-Inositol 2 g twice a day and forty-one patients received D-*chiro* inositol 0.6 g twice a day.**

Results: **The results of our study showed that the total number of oocytes retrieved did not differ in the two treatments groups. However, the number of mature oocytes was significantly increased in the *myo*-inositol group compared to D-*chiro*-inositol. Concurrently, the number of immature oocytes decreased in *myo*-inositol treated patients. Furthermore, the myo-inositol-treated group showed an increase in the mean number of top quality embryos and in the total number of pregnancies compared to the D-*chiro*-inositol-treated group.**

Conclusions: **Our data show that, in PCOS patients having a normal insulin response, *myo*-inositol treatment rather than D-*chiro*-inositol is able to improve oocyte and embryo quality during ovarian stimulation protocols.**

Key Words:

Myo-inositol, D-*chiro*-inositol, Oocyte quality, Embryo quality, Ovarian stimulation, ICSI cycles.

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of infertility due to menstrual dysfunction and it affects about 10% of women in childbearing age[1]. Its diagnosis is rather complex, and indeed diagnostic criteria changed over time. The most recent revision was performed during a consensus meeting sponsored by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) held in Rotterdam in 2003[2,3]. The outcome of the meeting defined that PCOS diagnosis should be based on three different factors: (1) oligo-anovulation, (2) hyperandrogenism (clinical or biochemical) and (3) the presence of 12 or more follicles in each ovary measuring 2±9 mm in diameter, and/or increased ovarian volume (>10 ml)[2,3].

Recently, many studies focused on both the impaired glucose tolerance, that affects 30 to 40% of patients with PCOS[4], and the insulin resistance, that affects 50 to 80%[5] of women with PCOS. Insulin plays a direct role in the pathogenesis of hyperandrogenemia: it acts synergistically with luteinizing hormone (LH) to enhance the androgen production of theca cells[6]. Furthermore, insulin is able to reduce circulating levels of sex hormone binding globulin (SHBG). Therefore, lower SHBG levels result in a relative increase of free testosterone[7]. Since the report by Burghen et al[8] that described the association between PCOS and hyperinsulinemia, it has become clear that this syndrome has major metabolic as well as reproductive morbidities. How-

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ever, PCOS pathogenesis is still unknown and so far the most promising treatments are insulin sensitizing agents[1,9].

Inositol is a polyalcohol classified as insulin sensitizer, and it is the second messenger of the insulin signalling[10]. Two different stereoisomers are currently used in the treatment of PCOS: *myo*-inositol (MI) and D-*chiro*-inositol (DCI)[11-15]. Both stereoisomers have an insulin-like action *in vivo* and they exert their function of insulin mediators as Inositolphosphoglycan (IPG)[16]. MI is the most abundant form of inositol in nature, while DCI is synthetized by an epimerase that convert MI to DCI. In particular, this reaction is insulin dependent[16], and insulin sensitive tissue such as liver and muscle have shown to be the main conversion sites[17]. Additional data also showed that in such tissues there is a correlation between insulin resistance and a reduced MI to DCI epimerization rate[18]. On the basis of these data, Authors suggest that a defect in tissue availability or utilisation of MI and/or DCI or mediators may contribute to insulin resistance[16,19].

A recent review clearly summarized the complex role the MI has in human reproduction[20]. Indeed, besides the positive effects on reducing insulin resistance[12,13,21] it has been shown that MI concentration in the follicular fluid directly correlates with oocytes quality[22]. Additional studies also showed that MI supplementation improves oocytes quality in PCOS patients[23].

In the present paper, we aim to compare the effect of MI and DCI supplementation in euglycemic PCOS patients undergoing ovulation induction for intracytoplasmic sperm injection (ICSI) procedure.

Materials and Methods

Patients

All patients treated in our *in vitro* fertilization (IVF) Department of infertility for a period of more than 12 months were asked to participate to the study.

A total of 84 women, aged <40 years, diagnosed with PCOS according to Rotterdam criteria[2,3] were included in this study. We excluded from the study patients that showed insulin resistance and/or hyperglycaemia.

ICSI procedures were suggested after evaluation of two different sperm samples of the male partner.

Patients were randomly assigned to receive either MI 2 g twice a day (43 subjects, group A) or DCI 0.6 g always twice a day (41 subjects, group B).

Both treatments were performed for 8 weeks before follicle stimulating hormone (rFSH) administration.

The Institutional Ethical Committee approved the protocol, and all patients gave a written informed consent before entering the study.

Controlled Ovarian Hyperstimulation

All patients underwent pituitary desensitization by subcutaneous (s.c.) administration of a gonadotropin releasing hormone (GnRH) agonist (Decapeptyl; Ipsen, Paris, France) from midluteal phase until the intramuscular (i.m.) administration of 10,000 IU human chorionic gonadotropin (hCG). Then, controlled ovarian hyperstimulation was performed in all patients by administration of rFSH (Gonal-F; Merck-Serono, Geneva, Switzerland). Starting dose was 150 IU per day. Patients were monitored by measuring the plasma concentration of 17β-Estradiol 2 17β-E_2 and the size of follicles on day 5 of the stimulation. The amount of gonadotropin administered was adjusted according to the individual response. The 10,000 IU hCG was injected i.m. in all patients when serum 17β-E_2 exceeded 200 pg per follicle and there were at least three follicles with a minimum diameter of 18 mm. Cycles were canceled if E_2 levels were >4,000 pg/mL, due to increased risk of ovarian hyperstimulation syndrome (OHSS).

ICSI Procedure

Since March 10, 2004 the Italian IVF law state that a maximum of three oocytes per patient could be injected, while spare mature oocytes were cryopreserved according to protocols described in previous studies[24]. Oocyte and sperm preparation for conventional ICSI procedure have been thoroughly described elsewhere[25]. Concerning ICSI, cumulus and corona radiata cells were immediately removed after retrieval by a short exposure to HEPES-buffered medium (Quinn's Advantage Hepes Medium; Sage IVF, Trumbull, CT, USA) containing 20 IU/mL hyaluronidase (Sage IVF) and gentle aspiration in and out of a Pasteur pipette and mechanically cleaned from the remaining surrounding cumulus cells by aspiration using a denuding pipette (Denuding Flexi-Pet; Cook, Brisbane, Australia) with a 170-130 mm diameter. The denuded oocytes were then assessed for their meiotic maturation status. In preparation for ICSI, oocytes with an extruded first polar body presumably at the

metaphase II stage (MII) were selected (in a maximum of three) for the fresh cycle and spare MII oocytes were cryopreserved, if required[26].

Luteal Phase

Intramuscular administration of 50 mg daily progesterone in-oil was started on the day of ovum pick-up, and treatment was performed daily until either a serum pregnancy test result was negative or an embryonic heart beat was sonographically confirmed.

Determination of Pregnancy States

A biochemical pregnancy was defined as a small and transitory increase in β-hCG levels. A clinical pregnancy was determined by the visualization of an embryo with cardiac activity at 6-7 weeks of gestation. Spontaneous abortion was classified as the loss of the pregnancy between the fifth and twelfth weeks of gestation.

Statistical Analysis

The statistical package SPSS Kit SigmaStat for Windows V2.03S (SPPS, Chicago, IL, USA) was used for data analysis. Baseline characteristics and ovulation induction (Table I) were analyzed using the unpaired Student' *t*-test. Ovum pick-up outcomes were analyzed using Wilcoxon' test; pregnancy rates were compared using Fisher exact test. Results with $p<0.05$ were considered to be significant.

Results

During the study period, 84 patients matching the inclusion criteria were randomized into two groups as previously described.

Group A (MI) consisted in 43 patients and Group B (DCI) consisted in 41. No differences were found between the two groups in mean age, Body Mass Index (BMI), and duration of infertility (Table I).

Total r-FSH units (1953.6 ± 397.5 *vs.* 2360.5± 301.9; $p<0.01$) and number of days of stimulation (11.1 ± 0.8 *vs.* 12.7 ± 1.1; $p<0.01$) were significantly reduced in the MI group. Furthermore, peak estradiol levels (2261.2 ± 456.6 *vs.* 2740.0 ± 396.7 pg/ml; $p<0.01$) at hCG administration were significantly lower in MI-treated versus DCI-treated patients (Table I).

No cycle was cancelled in group A, while in group B four cycles were cancelled due to estradiol peak >4000 pg/ml ($p=0.05$ Table I).

The total number of oocytes retrieved did not differ between the two groups, while the number of mature oocytes significantly differed, being 8.21± 2.39 in the MI group *vs.* 7.08 ± 2.67 in the DCI group ($p<0.05$, Table II). Concurrently, the number of immature oocytes retrieved was significantly lower in the MI group compared to DCI group (0.69 ± 0.64 *vs.* 2.23 ± 0.85; $p<0.01$, Table II)

Noteworthy, the number of grade 1 embryos was significantly increased by MI supplementation (1.64 ± 0.88 *vs.* 0.76 ± 0.43; $p<0.01$, Table II).

In compliance with the Italian IVF law, at maximum three oocytes per patients were injected. A total of 32 pregnancies were obtained (22 in group A *vs.* 10 in group B; $p<0.05$, Table III).

Discussion

In the present paper, we show that MI rather than DCI is able to improve oocyte and embryo quality in euglycemic PCOS patients. This is in

Table I. Characteristics and outcome of treated patients (Mean ± SD).

	MI	DCI	*P* value
No. of patients	43	41	
Age (yrs)	35.5 ± 3.2	36.5 ± 2.5	NS
duration of infertility (months)	50.3 ± 10.3	45 ± 15.6	NS
BMI (kg/m^2)	24.6 ± 8.4	25.3 ± 7.8	NS
PRL (ng/ml)	18.1 ± 2.9	19.3 ±2.4	NS
TSH (mIU/L)	1.7 ± 1.1	1.8 ±0.9	NS
Stimulation (days)	11,1 ± 0,8	12,7 ± 1,1	< 0.01
FSH IU administrated	1953.6 ± 397.5	2360.5 ± 301.9	< 0.01
17b-E2 levels on hGC administration (pg/ml)	2261.2 ± 456.6	2740 ± 396.67	< 0.01
No. Of cancelled cycles (E_2 > 4000 pg/ml)	0	4	0.05

Table II. Oocyte and embryo quality (Mean ± SD).

	MI	DCI	*P* value
No. of retrived oocytes	8.90 ± 2.84	9.32 ± 3.15	NS
No. of MII oocytes	8.21 ± 2.39	7.08 ± 2.67	< 0.05
No. of immature oocytes	0.69 ± 0.64	2.23 ± 0.85	< 0.01
Embryo grade 1	1.64 ± 0.88	0.76 ± 0.43	< 0.01

Table III. Pregnancy outcome.

	MI	DCI	*P* value
No. of pregnancies (%[a])	22 (51)	10 (24)	< 0.05
No. of biochemical pregnancies (%[b])	3 (14)	2 (9)	NS
No. of clinical pregnacies (%[b])	15 (68)	5 (22)	NS
No. of spontaneous abortion (%[b])	4 (18)	3 (14)	NS

a = versus patients number; b = versus total pregnancies.

line with previous findings by Papaleo et al, who showed that MI supplementation significantly reduced ovarian stimulation days and the IU of rFSH administrated compared to placebo[23].

The most common cause of IVF-ET failure is the reduced embryo quality and several factors, such as social-environmental, aging and/or pathological factors, can negatively affect it[27-30].

Ooocyte retrieved from PCOS patients are indeed characterized by poor oocyte quality[27,28] and, therefore, any treatment able to improve oocyte quality could be considered the "holy grail" for IVF procedures.

Recently, it has been shown that two molecules, normally produced by our body, MI and Melatonin are efficient predictors for oocyte quality and IVF outcomes: indeed, high concentration of both molecules positively correlates with high oocyte quality[22,31,32]. In particular, several clinical trials have shown that supplementation with MI, alone or in association with melatonin, is a practical approach able to improve oocyte quality and IVF outcomes in both PCOS patients and normal subjects[23,30,33].

Menstrual dysfunctions in PCOS patients are mainly caused by hyperandrogenism. In these patients, insulin sensitizer compounds have been shown to be able to normalize androgen levels in both obese and lean women[11-13,15,34]. In particular, both MI and DCI have been of interest for the scientific community[11-13,15,34].

Several studies aiming at identifying the cause of insulin resistance pointed out that the relative amount of both molecules is regulated by insulin. Indeed, insulin regulates the epimerization of MI into DCI in a dose dependent fashion. Furthermore, it was found that insulin resistance impairs MI to DCI epimerization altering the ratio between MI/DCI, resulting in higher MI levels[35,36].

These studies were performed on insulin sensitive tissues such as hepatic and muscular tissue, and no data are reported on ovarian tissue, that never becomes insulin resistant. Therefore, we could speculate that PCOS patients suffering of hyperinsulinemia likely present an enhanced MI to DCI epimerization in the ovary, leading to an alteration of MI/DCI ratio and probably to a MI depletion in the ovary. This MI depletion could eventually be responsible of the poor oocyte quality observed in these patients.

However, in order to prove this hypothesis and to fully understand the role of MI and DCI on ovarian function of PCOS patients, further studies need to be specifically performed on ovarian tissue.

In conclusion, in the present paper we were able to demonstrate that MI rather than DCI supplementation has a direct positive effect on oocyte and embryo quality.

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