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# The combined therapy myo-inositol plus D-chiro-inositol, rather than D-chiro-inositol, is able to improve IVF outcomes: results from a randomized controlled trial

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**Abstract**
*Purpose* The present study aims to investigate the effects of the combined therapy myo-inositol (MI) plus D-chiro-inositol (DCI) or D-chiro-inositol treatment in oocyte quality.
*Methods* Polycystic ovary syndrome (PCOS) women undergoing IVF-ET were treated with myo-inositol combined with D-chiro-inositol in the physiological ratio (1.1 g myo-inositol plus 27.6 mg of D-chiro-inositol; INOFOLIC® combi Lo.Li.pharma) or D-chiro-inositol alone (500 mg; Interquim, s.a., Barcelona, Spain) to evaluate the umber of morphological mature oocytes, total International Units (IU) of recombinant FSH administered and the number of grade 1 embryos.
*Results* The data clearly showed that only the combined therapy was able to improve oocyte and embryo quality, as well as pregnancy rates, in PCOS women undergoing IVF-ET.
*Conclusion* The present paper further supports the hypothesis that MI plays a crucial role in the ovary in PCOS women. In particular, due to the physiological role played by MI and DCI, the combined therapy should represent a better choice.

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## Introduction

Assisted reproductive technologies (ART) have a 30-year-old history, and all studies performed so far agree upon the importance of identifying oocyte quality as a main predictor of a positive outcome [1, 2]. The most important factor affecting oocyte quality is a woman's age. However, nowadays many couples choose to delay parenthood. Thus, the mean age of women who become first-time mothers in the USA has increased over the last 36 years from 21.4 to 25.0 years [3].

To help more people fulfill their parental desire, several studies have focused on identifying compounds that are able to maintain and/or improve oocyte quality [4]. Among the different factors identified, two have been shown to be highly predictive: myo-inositol (MI) and melatonin (M). In particular, MI and M concentrations in follicular fluid directly correlate with good oocyte quality. Furthermore, a direct correlation between MI and M concentrations has also been found with regard to embryo quality [5, 6].

As a consequence, both MI and M have already found application in several clinical trials aiming to study MI and M supplementation as an effective treatment to improve oocyte and embryo quality in polycystic ovary syndrome (PCOS) patients [7–9]. PCOS patients were chosen as therapeutic targets because they typically suffer from reduced oocyte quality [10, 11].

The results of those studies clearly showed that MI and M supplementation effectively improved oocyte and embryo quality [7–9] in PCOS patients. Therefore, MI and M supplementation could be considered to positively

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predict an increased chance of achieving a pregnancy in PCOS patients who undergo ART.

Inositol is a polyalcohol existing as nine different isomers [12], two of which myo-inositol (MI) and D-chiro-inositol (DCI) have been identified as insulin mediators [13] and have been used as insulin sensitizers in the treatment of PCOS [14–19]. Although the two molecules have similar structure, it has been shown that MI is more effective than DCI in the management of PCOS patients [14–18, 20, 21]. Indeed, results are consistent throughout several trials and in particular it has been shown that MI is able to induce menstrual regularity while DCI does not [19, 22]. Furthermore, it has been shown that only MI is able to improve oocyte quality in PCOS patients [23].

MI is the most abundant isoform of inositol in nature, while DCI is synthesized by an epimerase that converts MI to DCI. In particular, this reaction is insulin dependent [24] and in each tissue it is possible to identify a unique ratio between the two molecules [25]. The reason for this unique ratio is that it is linked, very likely, to the specific biological function these molecules have. Indeed, following insulin stimulation, inositol(s)-second messengers are produced; while DCI-based second messengers promote glycogen synthesis, the second messengers based on MI regulate glucose intake and mediate FSH signaling [26]. Tissue-specific ratio well resemble the different functions of these molecules; higher DCI inositol concentrations (always lower than MI) are present in the liver, muscle and fat, while extremely low DCI concentrations are present in tissues such as brain, heart and kidney [25].

Recently, a more detailed study of the inositol system has led to the identification of a physiological blood ratio of the two molecules and therefore an ideal and more effective treatment has been designed [27].

Since it has been shown that MI supplementation alone outperforms DCI during IVF protocols [23], in the present study we aim to assess whether the combined therapy still outperforms DCI alone and whether MI-DCI therapy still retains all the beneficial effects already proven for MI [7, 20].

## Materials and methods

### Patients

All patients treated in our IVF department over a period greater than 12 months were asked to participate in the study. This included a total of 100 women having a BMI $< 28$ and FSH $< 10$ IU/L with a diagnosis of PCOS according to Rotterdam 2003 and a normal uterine cavity.

The study excluded patients diagnosed with advanced stage (III or IV) endometriosis and those classified as poor responders or as suffering from premature ovarian failure. ICSI procedures were suggested after the evaluation of two different sperm samples from the male partner.

Patients were randomly assigned to a block of ten by a computer-generated program to receive either MI 550 mg and DCI 13.8 mg orally twice a day (INOFOLIC® combi, soft gel, Lo.Li. Pharma Roma, Italy; patented) (47 subjects) or DCI 500 mg orally twice a day (Interquim, s.a., Barcelona, Spain) (53 subjects).

The key to the coding of the treatments was kept by the Lo.Li. Pharma. Both the participants and the research team were blinded. The randomization code was not broken until the completion of the study.

Both treatments were performed for 12 weeks before rFSH administration and throughout pregancy. The institutional review board approved the protocol, and all patients gave written informed consent before entering the study.

The trial was registered on clinicaltrials.org (NCT1338844).

### Dropout

One of the patients who was enrolled and assigned to the MI-DCI treated group decided to quit the IVF procedure due to personal reasons.

### Controlled ovarian hyperstimulation

All patients underwent pituitary desensitization by subcutaneous (s.c.) administration of a GnRH agonist (Decapeptyl; Ipsen, Paris, France) from the midluteal phase of the menstrual cycle until the day of intramuscular (i.m.) administration of 10,000 IU of hCG. Following down-regulation, controlled ovarian hyperstimulation was performed in all patients by the administration of rFSH (Gonal-F; Merck-Serono, Geneva, Switzerland). The starting dosage of rFSH was 150 IU s.c. per day. Patients were monitored by measuring their plasma concentration of estradiol ($E_2$) and the size of their follicles via transvaginal sonography, starting on day 5 of stimulation. The amount of gonadotropin administered was adjusted according to each patient's individual response. 10,000 IU hCG was injected i.m. in all patients when the serum $E_2$ exceeded 200 picograms (pg) per mature 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 an increased risk of ovarian hyperstimulation syndrome (OHSS).

### ICSI procedure

Since 10 March 2004, Italian IVF law states that a maximum of three oocytes per patient can be injected with sperm, while spare mature oocytes are to be cryopreserved

according to protocols described in previous studies [28]. The preparation of oocytes and sperm for conventional ICSI procedures has been thoroughly described elsewhere [29]. 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) containing 20 IU/mL hyaluronidase (Sage IVF) and by gentle aspiration in and out of a Pasteur pipette. This was followed by mechanical cleaning 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 subsequently 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 (with a maximum of three) for the fresh cycle, while spare MII oocytes were cryopreserved, if required [30].

### Luteal phase

Daily intramuscular administration of 50 mg of progesterone in oil was started on the day of ovum pick-up, and this treatment was continued daily until either a serum pregnancy test returned as negative or an embryonic heart beat was sonographically confirmed.

### Outcome measures

Primary outcomes: the number of morphologically mature oocytes, total IU of recombinant FSH administrated and the number of grade 1 embryos.

Secondary outcomes: $E_2$ levels before hGC injection, the number of degenerated oocytes, maturation rate, fertilization rate and the number of embryo transferred.

### Statistical analysis

The statistical package SPSS Kit SigmaStat for Windows V2.03S was used for data analysis. Baseline characteristics and ovulation induction (Tables 1, 2) were analyzed using the unpaired Student's *t* test. Ovum pick-up outcomes were analyzed using the Wilcoxon test; pregnancy rates were compared using Fisher's exact test. Results with $P < 0.05$ were considered to be statistically significant.

## Results

Since it is universally accepted that the age of 35 years is a milestone for women's reproductive function, we decided to divide and analyze our dataset into two: patients equal to or younger than 35 years and patients older than 35 years. Results obtained are shown in Table 1 (women $\leq$ 35 years) and Table 2 (women $>$ 35 years).

### FSH administration and estradiol levels

The ovarian stimulation protocol resulted in greater efficiency in the MI-DCI group (INOFOLIC® combi) for both younger and older age categories, (Tables 1, 2). Indeed, less IU of rFSH was administered in the MI-DCI group (INOFOLIC® combi); in particular, in the $\leq$35 years group, the difference was statistically significant (Table 1, upper panel): 1,569.02 $\pm$ 497.12 in the MI-DCI group (INOFOLIC® combi) versus 1,899.21 $\pm$ 618.17 in the DCI group. Concomitantly, $E_2$ levels before hCG administration were lower in the MI-DCI group (Tables 1, 2), although a statistically significant difference was achieved only in the group $>$35 years (Table 2, upper panel): 2,185.09 $\pm$ 409.08 in the MI group versus 2,519.85 $\pm$ 788.49 in the DCI group.

### Number and quality of oocytes

Concerning oocyte number, patients respond differently according to their age category. No differences were found in the number of oocytes retrieved between the groups in the $\leq$35 age category (Table 1). On the contrary, in the $>$35 age category, the number of oocytes retrieved was higher in the DCI group (Table 2): 8.35 $\pm$ 3.21 in the MI-DCI group versus 10.75 $\pm$ 5.23 in the DCI group.

Oocyte quality was analyzed taking into account the number of mature oocytes (MII), the number of immature oocytes and the presence of degenerated oocytes (VG-DEG). The data showed that MI-DCI treatment reduced the number of VG-DEG in both the younger and the older age groups ($\leq$35: 1.04 $\pm$ 1.15 vs. 1.82 $\pm$ 1.55; $>$35 1.00 $\pm$ 0.91 vs. 1.45 $\pm$ 0.89).

### Number and quality of embryos

Higher oocyte quality likely had a direct effect on the fertilization rate, as well as the number and quality of embryos. Indeed, in the $\leq$35 category, the fertilization rate was 0.75 $\pm$ 0.24 in the MI-DCI treated group, while it was 0.58 $\pm$ 0.29 in the DCI treated group ($P < 0.05$). The increased fertilization rate directly influenced the number of transferred embryos; in particular, 2.22 $\pm$ 0.74 embryos were transferred in the MI-DCI group, while 1.67 $\pm$ 0.85 was available for transfer in the DCI treated group ($P < 0.05$). Concerning embryos, the difference between the two groups was not only related to embryo number, but extended to embryo quality as well. Indeed, the MI-DCI treated group had higher embryo quality compared to DCI treated patients (0.96 $\pm$ 0.83 vs. 0.7 $\pm$ 0.73) ($P < 0.05$).

**Table 1** Effect of the combined therapy myo-inostitol plus D-chiro-inositol or D-chiro-inositol supplementation on hormonal levels, oocyte and embryo quality for women undergoing ICSI, younger than 35 years old

| | ≤35 years | | | | | | |
|---|---|---|---|---|---|---|---|
| | MI-DCI (23) | | | DCI (33) | | | *P* value |
| | Mean | SD | 95 % CI | Mean | SD | 95 % CI | |
| FSH | 1,569.02 | 497.12 | 1,354.05–1783.99 | 1,899.21 | 618.17 | 1,680.02–2,118.41 | 0.04 |
| $E_2$ | 2,230.09 | 827.57 | 1,872.22–2587.95 | 2,537.94 | 860.19 | 2,232.93–2,842.95 | NS |
| | MI (23) | | | DCI (33) | | | *P* value |
| | Mean | SD | 95 % CI | Mean | SD | 95 % CI | |
| No. of oocytes | 9.91 | 4.85 | 7.82–12.01 | 10.79 | 4.66 | 9.14–12.44 | NS |
| MII | 7.91 | 4.51 | 5.96–9.86 | 8.00 | 3.92 | 6.61–9.36 | NS |
| VG-DEG | 1.04 | 1.15 | 0.55–1.54 | 1.82 | 1.55 | 1.27–2.37 | 0.04 |
| Embryo transfer | 2.22 | 0.74 | 1.90–2.54 | 1.67 | 0.85 | 1.36–1.97 | 0.01 |
| Mat rate[a] | 0.80 (181) | 0.15 | 0.73–0.86 | 0.74 (294) | 0.19 | 0.68–0.81 | NS |
| Fert rate[a] | 0.75 (51) | 0.24 | 0.64–0.85 | 0.58 (56) | 0.29 | 0.47–0.68 | 0.03 |
| EG1 rate[a] | 0.96 (49) | 0.83 | 0.60–1.31 | 0.73 (39) | 0.73 | 0.44–0.96 | 0.001 |

*MI-DCI* combined therapy myo-inositol plus D-chiro-inositol, *DCI* D-chiro-inositol, *MII* mature oocytes, $E_2$ Estradiol levels before hCG administration, *VG-DEG* immature oocytes and degenerated oocytes

[a] Values are tabled as percentage and absolute numbers are in brackets

**Table 2** Effect of the combined therapy myo-inostitol plus D-chiro-inositol or D-chiro-inositol supplementation on hormonal levels, oocyte and embryo quality for women undergoing ICSI, older than 35 years old

| | >35 years | | | | | | |
|---|---|---|---|---|---|---|---|
| | MI ($N = 23$) | | | DCI ($N = 20$) | | | *P* value |
| | Mean | SD | 95 % CI | Mean | SD | 95 % CI | |
| FSH | 1,906.96 | 770.59 | 1,573.73–2,240.19 | 2,170.58 | 694.44 | 1,845.57–2,495.58 | NS |
| $E_2$ | 2,185.09 | 409.08 | 2,008.19–2,361.99 | 2,519.85 | 788.49 | 2,150.83–2,888.87 | 0.05 |
| | MI ($N = 23$) | | | DCI ($N = 33$) | | | *P* value |
| | Mean | SD | 95 % CI | Mean | SD | 95 % CI | |
| No. of oocytes | 8.35 | 3.21 | 6.96–9.74 | 10.75 | 5.23 | 8.30–13.20 | 0.05 |
| MII | 6.91 | 2.26 | 5.94–7.89 | 8.35 | 5.19 | 5.92–10.78 | NS |
| VG-DEG | 1.00 | 0.91 | 0.61–1.39 | 1.45 | 0.89 | 1.03–1.87 | 0.05 |
| Embryo transfer | 2.00 | 0.74 | 1.68–2.32 | 2.20 | 0.77 | 1.84–2.56 | NS |
| Mat rate[a] | 0.84 (162) | 0.10 | 0.80–0.88 | 0.76 (163) | 0.13 | 0.69–0.82 | 0.04 |
| Fert rate[a] | 0.67 (46) | 0.25 | 0.56–0.77 | 0.73 (43) | 0.26 | 0.61–0.85 | NS |
| EG1 rate[a] | 0.90 (41) | 0.80 | 0.63–1.37 | 0.68 (30) | 0.80 | 0.44–1.13 | 0.02 |

*MI-DCI* combined therapy myo-inositol plus D-chiro-inositol, *DCI* D-chiro-inositol, *MII* mature oocytes, $E_2$ Estradiol levels before hCG administration, *VG-DEG* immature oocytes and degenerated oocytes

[a] Values are tabled as percentage and absolute numbers are in brackets

In the patients >35 years old, although there was no difference between the fertilization rate and the number of transferred embryos, there was a significant difference in embryo quality. In fact, the MI-DCI treated patients showed an increase in the number of high-quality embryos compared to the DCI treated group ($0.9 \pm 0.8$ vs. $0.68 \pm 0.8$) ($P < 0.05$).

## Discussion

In the present paper, we were able to show that combined therapy MI-DCI, rather than DCI, was able to improve oocyte quality in PCOS women undergoing assisted reproductive technology (ART). When we set out to conduct this study, our original intent was to include a higher

number of participants. However, due to a recently published paper, demonstrating a negative impact of DCI supplementation at the dosage of 2.4 g per day in IVF [31], for ethical reasons, we decided to close the study, suspend the DCI supplementation and switch all of our IVF patients to the combined treatment.

Although in recent years there has been a marked improvement in IVF technologies, the average success rate is approximately 30 %. Therefore, every treatment with the potential to improve the outcome of IVF should be considered a predictive factor and should be included in ART protocols.

The link between inositol(s) and life can be dated to the prebiotic era. Indeed, several evidences suggest that inositol was one of the molecules present in the primordial soup [32].

Indirect evidence of this can be easily found. Indeed, inositol-based molecules are the most common second messenger and basically are defined as a new biological code that needs to be translated [33].

In the inositol millenary history, several milestones can be placed: (1) inositol isolation, by Schender [34], (2) identification as calcium second messenger, (3) identification as insulin second messenger [35] (for review). At this point the big leap in understanding the role played by myo-inositol in reproduction was taken by Chiu and Tam [36]. The authors identified myo-inositol as a key molecule for supporting pregnancy. Independently, Nestler and coworkers were able to identify some beneficial effects obtained by PCOS patient treated with DCI. A few years later, Chiu and coworkers linked in a tighter way myo-inositol to reproduction. Indeed, they were the first to show that in myo-inositol concentration in the follicular fluid was crucial to have high-quality oocyte [5]. Based on these evidences, our group started studying myo-inositol biology and to apply myo-inositol and decided to evaluate its effect in PCOS clinical practice.

The beneficial effects of inositol in reproduction are well documented [37–39]. In particular, data on ovulation induction in PCOS patients were available for the two main isomers, MI and DCI [16–19, 40]. In particular, only two trials were performed on DCI and both of them focused on "one-time" ovulation rather than on menstrual regularities. Furthermore, results were not confirmed when DCI dosage was doubled [22]. This could be explained with the theory proposed by Carlomagno et al. [41] and by the data obtained by Isabella et al. in 2012. In their study, Isabella et al. demonstrated that an increased amount of DCI (2.4 g per day) worsens ovarian response [42].

The real breakthrough with MI in the IVF arena was achieved in 2009 when it was shown that MI supplementation was able to improve poor oocyte quality in PCOS patients [7]. Since then, several trials have further supported MI effectiveness [20, 23, 43, 44]. Indeed, in these studies about 80 % of the oocytes retrieved from MI treated patients were of high quality, while it was only 66 % for the control group.

MI and DCI are two different molecules that by "accident" share name and structure, but as already mentioned these two molecules have different biological functions.

In the present study, we tested a new formulation that provided both molecules in a physiological ratio. In particular, we demonstrated that the combined therapy retained all the beneficial effect of MI treatment alone. In particular, the supplementation of both molecules in a physiological ratio exerts a greater beneficial effect on the metabolic imbalance that affects PCOS women and may better prevent the development of pathologies like gestational diabetes [45, 46].

We demonstrated that only the MI-DCI treated group was characterized by an increase in embryo quality. Notably, stimulation protocols in MI-DCI treated patients were more efficient; indeed, lower dosages of FSH were administered for a shorter period of time. As expected, this, in combination with better embryo quality, directly increased pregnancy rates [1, 47].

Overall, data obtained from previous works [5, 7, 23] and from the present study clearly indicate the pivotal role played by MI in the ovary to obtain high-quality oocytes. In particular, MI supplementation could be considered a predictive factor in both "natural" and assisted reproduction.

The ratio of MI/DCI is regulated by an epimerase that converts MI into DCI. Larner [25] showed that each organ has a specific MI/DCI ratio. Furthermore, it was shown that insulin could stimulate enzymatic activity leading to an increased MI to DCI conversion rate [48]. These data led us to hypothesize that a "DCI paradox exists in the ovary" [41]. Data present in literature show that by providing both molecules in a physiological ratio, hormonal and metabolic imbalances are treated much more quickly compared to MI alone. The present data broaden our knowledge, showing that also IVF protocol might take advantage of the combined therapy.

The fact that each organ has its own specific DCI/MI ratio suggests that the balance between the two isomers has an important biological function. Therefore, since it was demonstrated that the human body cannot catalyze the conversion of DCI to MI [48–50], DCI supplementation alone should be avoided during IVF protocols.

**Conflict of interest** None.

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