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ORIGINAL ARTICLE

High dose of D-chiro-inositol improves oocyte quality in women with polycystic ovary syndrome undergoing ICSI: a randomized controlled trial

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ABSTRACT

The aim of this study was to evaluate the effect of two doses of D-chiro-inositol (DCI) in combination with Myo-inositol (MYO) on the oocyte quality (OQ) of women with polycystic ovarian syndrome (PCOS) undergoing intracytoplasmic sperm injection (ICSI). *Methods:* This was a controlled, randomized, double-blind, parallel group study on 172 oocytes from 11 women. The study compared the effect of two MYO-DCI formulations given over 12 weeks on OQ. Five women received 550 mg of MYO + 300 mg of DCI daily (high DCI content group), while 6 women were given a daily dose of 550 mg of MYO with the only 27.6 mg of DCI (low DCI content group). *Results:* According to a multivariate analysis using linear mixed effect models, high doses of DCI have a positive influence on the quality of the cytoplasm of the oocyte ($\beta = 1.631$, $\chi^2 = 7.347$, d.f. = 1, $p = .00672$). Zona pellucida, plasma membrane, cytoplasm, and sperm reception have also been improved with any combination of MYO/DCI by decreasing testosterone or improving insulin sensitivity, regardless of age and body mass index. *Conclusion:* The combination of MYO with high doses of DCI improved oocyte cytoplasm quality in women with PCOS undergoing ICSI.

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D-chiro-inositol; PCOS; ICSI; oocyte quality

Introduction

In women with polycystic ovarian syndrome (PCOS), reproductive improvements have been reported using inositol in various forms or combinations [1]. However, Myo-inositol (MYO) supplements alone were not sufficient to improve oocyte maturation, embryo quality or pregnancy rate [2], and there is controversy about the optimal dose of D-chiro-inositol (DCI) [3]

In a recent study, our group has observed that the combination of MYO-DCI at high doses of DCI improves the pregnancy rate in relation to the physiological ratio without differences in oocyte maturation or embryo quality [4]. The purpose of the current study is to determine if the quality of the oocytes will be improved according to specific oocyte quality (OQ) markers.

Materials and methods

Study design

This study was a double-blind, randomized clinical trial (RCT) conducted from February 2016 to April 2017 and performed in accordance with the guidelines of the Helsinki Declaration and Good Clinical Practice. The volunteers were randomly assigned to one of two groups according to a randomization scheme generated by a computer program (SIGESMUÂ®). To maintain blinding, the investigator received a treatment allocation number for each subject.

Subjects

Inclusion criteria: PCOS women aged 18–40 years according to the Rotterdam criteria [5] with a body mass index (BMI) <30 who have undergone an intracytoplasmic sperm injection (ICSI)

Exclusion criteria: Contraindications for ICSI, adrenal hyperplasia, hyperprolactinemia, thyroid disease, severe endometriosis, poor responders, and severe male factor.

Methods

Eleven PCOS women were randomized to receive oral soft gelatin capsules of 550 mg MYO + 150 mg DCI twice daily (3.6:1) (high-DCI concentration group) or 550 mg MYO + 13.8 mg DCI twice daily (40:1) (low DCI concentration group) for 12 weeks until the day of the ovarian puncture. Intake of other vitamins or antioxidants was not permitted during the study, with the exception of folic acid (400 mg/day), which was provided to all patients.

Ovulation stimulation was started according to a specified protocol using the dose of 150 units FSH for 5 d. After this initial stimulation, each patient was treated individually according to her response, and the cycle was controlled using a GnRH antagonist according to previously published criteria [4].

Ethical approval

The Granada Ethics and Clinical Research Committee approved the study. The patients signed a declaration of consent informing them about the procedure and possible risks of the study.

Outcomes

The primary result was the OQ. Seven specific morphological markers measured the OQ. The evaluation of the OQ was

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performed on the same day as the ovarian puncture was performed and each variable was assessed from 0 to 10 simultaneously by 2 embryologists, each variable receiving the average score of the 2 observers.

Oocyte quality markers

1. Zona pellucida: the highest score was assigned to the normal size and density.
2. Perivitelline space: the highest score was assigned to oocytes of sufficient size and morphology.
3. First Polar Body: normal size, smooth surface, and lack of fragmentation getting the highest score.
4. Plasma Membrane: This variable evaluates the elasticity of the membrane during injection, giving the highest score if it had optimal elasticity when inserting the injection needle.
5. Cytoplasm: the highest was assigned to cytoplasm without anomalies (centrally located granular cytoplasmic granulations, inclusions, smooth endoplasmic reticulum aggregates, vacuoles).
6. Sperm reception: This variable evaluates the displacement of the sperm as soon as it has been deposited in the oocyte and after the microinjection needle has been pulled out, giving the maximum score if the sperm has not moved away from the deposition site.
7. Injection Cone: This variable evaluates the printing remaining in the oocyte when the microinjection needle is removed, giving the highest score when the printing was more visible.

Statistical analysis

The differences between the OQ markers as a function of the DCI dose were studied by the Student's *t*-test for each marker, considering each oocyte as a sample element. To graphically observe the differences in the values of each OQ marker, we obtained a box and whisker plot for each marker.

The influence of other variables on OQ was investigated using a linear mixed-effect model since oocytes were not independent when measured on the same female. In addition, the assumptions of normality and multicollinearity were tested before the analysis for each of the linear models. Normality was tested by graphical analysis of the Q-Q plots and the multicollinearity of the independent variables was evaluated using the Variance Inflation Factor. For all models, pseudo-R2 coefficients were estimated for marginal (variance explained by fixed effects) and conditional (variance explained for the complete model). The beta coefficients of the fixed effects were estimated, as well as their Chi-square value, their degrees of freedom, and the *p*-value.

Statistical analyses were performed using R 3.5.1. [6] and the packages car [7], lme4 [8], and MuMIn [9].

Results

At baseline, no differences were found between the two groups. At the end of the ICSI cycle, 172 oocytes from 11 patients were analyzed. Total testosterone, glucose and insulin levels, HOMA-IR were similar in both groups (Table 1), number of MII oocytes and percentage of good quality embryos were also similar in both groups. Pregnancy was achieved in four patients in the high DCI group and in one patient in the low DCI group ($p = .036$).

Table 1. Variance Inflation Factors for each variable in each linear model according to dependent variable.

OQ marker	Treatment	Age	BMI	Dif. HOMA-IR	Dif. Testosterone
Zona Pellucida	1.3652	1.7236	1.6346	1.4865	1.1886
Perivitelline space	1.3475	1.7855	1.6632	1.5559	1.1980
Polar Body 1	1.3479	1.7840	1.6629	1.5543	1.1978
Plasma membrane	1.3480	1.7838	1.6628	1.5541	1.1977
Cytoplasma	1.3633	1.7298	1.6389	1.4939	1.1893
Sperm reception	1.3551	1.7583	1.6544	1.5263	1.1933
Injection cone	1.3467	1.7883	1.6638	1.5589	1.1986

For the OQ markers, the high dose of DCI gave better results in the cytoplasm, perivitelline space, plasma membrane, and cone injection. While, in the case of zona pellucida, sperm reception and PB1 were similar between doses of DCI (Figure 1; Table 2). The results of the analysis of the inflation factor by the variance (Table 3) show the absence of relevant colinearities.

According to a multivariate analysis using linear mixed effect models, high doses of DCI have a positive influence on the 'cytoplasm' ($\beta = 1.631$, χ^2 value = 7.42, d.f.=1, $p = .00645$).

Regardless of the dose of DCI, the decrease in HOMA-IR improves the 'cytoplasm' ($\beta = -1.832$, χ^2 value = 12.283, d.f.=1; $p = .0005$) and the 'zona pellucida' ($\beta = -1.377$, χ^2 value = 6.571, d.f.=1; $p = .0104$). The decrease in testosterone improves the 'plasma membrane' ($\beta = 0.511$, χ^2 value = 5.126, d.f.=1, $p = .0151$) and the 'sperm reception' ($\beta = 0.376$, χ^2 value = 5.763, d.f.=1, $p = .0164$).

Discussion

The primary results of this study show that a higher dose of DCI than recommended [10]. increases OQ, especially the quality of the cytoplasm, in women with PCOS who undergo ICSI. To our knowledge, this is the first RCT to study the effect of different doses of DCI on OQ.

An earlier study showed that pregnancy rates with the non-physiological MYO/DCI ratio were higher without improving oocyte maturation or embryo quality. Two explanations were proposed: firstly, the involvement of DCI in embryonic implantation and development; secondly, an effect of DCI on other variables of OQ, which go unnoticed in the classifications that are generally made. In this regard, the aim of the present study was to study in detail the impact of a higher DCI dose on specific OQ makers [4].

The main strength of the present study is the combination of analyzes of seven specific OQ markers. The knowledge about the morphological markers of OQ is low. An optimal mature oocyte is one that has a rounded shape, with a clear and uniform zona pellucida about 20 µm thick, with a translucent cytoplasm, free of inclusions and a polar body of appropriate size and shape [11,12].

However, the current state of knowledge about the morphological parameters of the oocyte does not allow to establish a clear correlation with the results of reproduction. For this reason, we still cannot rely on an OQ score as we do for the quality of embryos, although it is becoming increasingly apparent that they should be included in the future as more scientific evidence exists.

The main weakness of this study relates to two points: the subjectivity of the OQ score and the fact that it is part of the RCT. At our center, we have used a number of morphological OQ markers that have not previously been published and that we routinely use in the final report submitted to each patient

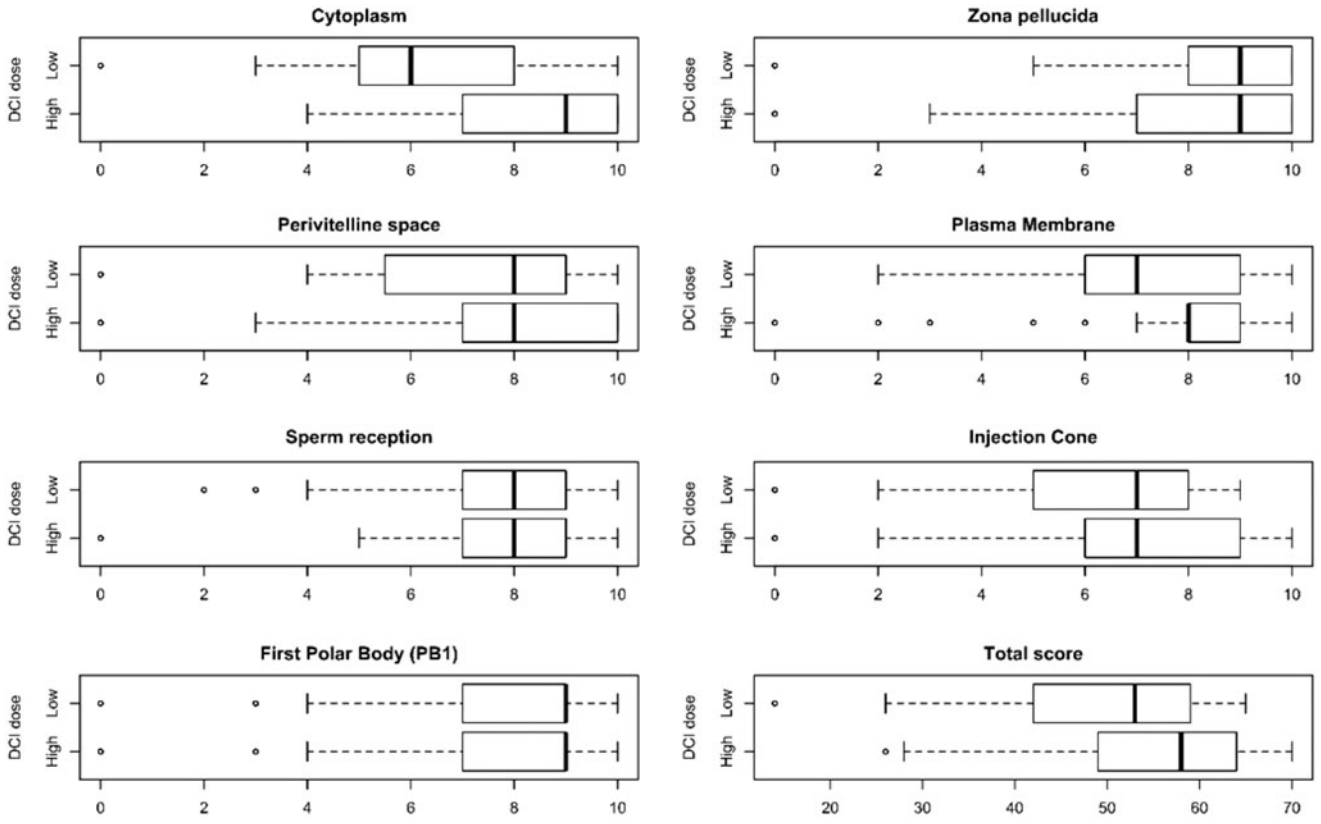

Figure 1. Box and whisker plot for OQ markers.

Table 2. Hormonal parameters.

Hormonal parameters	High-DCI concentration group	Low-DCI concentration group	*p* Value
Insulin at baseline (μUI/ml)	7.7 ± 1.1	6.81 ± 1.6	NS
Glucose at baseline (mg/dl)	92.4 ± 9.2	96 ± 22	NS
HOMA-IR at baseline	1.82 ± 0.67	1.62 ± 0.6	NS
Testosterone at baseline (ng/ml)	0.56 ± 0.11	0.69 ± 0.21	NS
Insulin at end (μUI/ml)	6.81 ± 3.26	7.16 ± 1.84	NS
Glucose at end (mg/dl)	88 ± 4.6	84.65 ± 7.84	NS
HOMA-IR at end	1.47 ± 0.79	1.49 ± 0.43	NS
Testosterone at end (ng/ml)	0.42 ± 0.16	0.62 ± 0.3	NS

Table 3. Means of oocyte quality indicators by groups.

	MEAN (μ)			
OQ marker	High-DCI group	Low-DCI group	μ1 to μ2, 95% CI	*p* Value
Zona Pellucida	8.151	8.687	[−1.243 to0.17]	0.9321457
Perivitelline space	7.767	7.051	[−0.123 to1.557]	0.04698271
Polar Body 1	7.767	7.798	[−0.71 to 0.648]	0.5357245
Plasma membrane	7.932	6.990	[0.28 to1.603]	0.00280059
Cytoplasm	8.521	6.061	[1.885 to3.035]	6.57E − 15
Sperm reception	7.795	7.646	[−0.412 to0.708]	0.3011837
Injection cone	7.014	6.121	[0.12 to1.665]	0.01194921

Confidence intervals at 95% and *p*-value of the Student's *t*-test to a tail.

after the ICSI trial. Since this is a special assessment, these OQ markers were not included in the first multicenter RCT [4].

As we have already shown in the original RCT, there is still confusion about the DCI dose that may be appropriate in women with PCOS, and we still ignore the cellular mechanisms underlying the improvements seen in this study underlying the OQ. The combination of MYO/DCI in a ratio of 40:1 was recommended as this is the physiological blood ratio of the two molecules. However, it was thought that the total dose of DCI may be more important than the ratio [13].

In the markers analyzed, we emphasized the quality of the cytoplasm, with a significant improvement with higher doses of DCI. Based on multivariate analysis using a mixed-effects linear model, there is strong evidence that the positive effect of high DCI on cytoplasmic mass is increased by 1.6 units compared to low DCI ($\beta = 1.571$, $\chi^2 = 7.420$, d.f.=1, $p = .00645$). Our data are consistent with the assumption that severe cytoplasmic changes, such as the presence of centrally located granular cytoplasmic granulations, inclusions, smooth endoplasmic reticulum aggregates, or excessive vacuolization, could affect embryonic development and its implantation potential. Furthermore, this support the hypothesis that the effects ofextracytoplasmic changes do not appear to correlate with embryo quality or implantation rates [14].

The elasticity of the plasma membrane and the printing of the injection cone are other markers apparently improved with a high DCI. However, the multivariate analysis does not show these differences and trials with more participants will likely be necessary.

On the other hand, we have also observed that some markers (zona pellucida, plasma membrane, cytoplasm, and sperm reception) have been improved with any combination of MYO/DCI by decreasing testosterone or improving insulin sensitivity. With regard to the oocyte plasma membrane, we observed that it had improved with the decrease of testosterone, regardless of the dose of DCI. The lower elasticity of the plasma membrane during the ICSI procedure can lead to oocyte damage or death, as well as reproductive consequences, as it 'allows the embryo to hatch and implanted in it.' [15]. Therefore, the quality of the plasma membrane could also improve the implantation of embryos, which is one of the possible results of a high-dose DCI.

The benefits of DCI over OQ could be explained by mechanisms that affect the ovum itself or the composition of the follicular fluid. It is known that the OQ is not only influenced at the genomic level, but also by the follicular microenvironment, which mainly affects the maturity/cytoplasmic quality [16–21].

At the oocyte level, perhaps because DCI is the substrate of phosphatidylinositol-3-kinases (PI3Ks), an enzyme involved in oocyte activation and in the survival and activity of granulosa cells on which OQ depends, but which may equally be important in the oocyte itself. Studies using genetically modified mouse models revealed that the PI3K signaling pathway within oocytes preserves and activates the original follicles [22]. In addition, based on an in vivo study in mice, the intracellular PI3K pathway is likely to be superfluous for the regulation of meiotic resumption of oocytes and early embryonic development [23,24].

In addition to the need for further studies with broader populations, the use of specific and homogeneous OQ markers is important. To adequately overcome the doubts about the intracystic mechanisms of DCI action, we believe that more research is required on the intracellular metabolism of inositol and each of its isoforms.

Conclusion

In conclusion, the use of MYO/DCI improves OQ by reducing testosterone and increasing insulin sensitivity. The combination of MYO-DCI at high doses of DCI improves the cytoplasmic quality of the oocyte with respect to its physiological concentration. These results highlight the importance of DCI supplementation in women with PCOS undergoing ICSI.

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Details of ethics approval

The study was approved at each clinical site. The Ethical Committee of the principal researcher (N. Mendoza) was the Ethics Committee of Granada (CEI, Consejería de Salud).

Disclosure statement

MP Diaz-Ropero, J Fonollá and M Olivares are workers of Biosearch Life, company that produces DCI from carob fruit. The rest of the authors declare that they have no competing interests.

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