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Can high levels of D-chiro-inositol in follicular fluid exert detrimental effects on blastocyst quality?

K. RAVANOS[1], G. MONASTRA[2], T. PAVLIDOU[1], M. GOUDAKOU[1], N. PRAPAS[1]

[1]IAKENTRO Fertility Centre, IVF laboratory, Thessaloniki, Greece
[2]Department of Experimental Medicine, "Sapienza" University, Rome, Italy

Abstract. – OBJECTIVE: It was previously shown that higher concentrations of myo-inositol in human follicular fluid improve oocyte and embryo quality, whereas D-chiro-inositol seems to worsen oocyte quality and ovarian response in polycystic ovary syndrome patients. Our study was the first one aiming to test whether different myo-inositol and D-chiro-inositol concentration in follicular fluids correlate with blastocyst quality in healthy young women.

PATIENTS AND METHODS: Eight egg donors and eleven couples undergoing *in vitro* fertilization, were involved in a prospective observational study. Myo-inositol/D-chiro-inositol ratio was calculated in the follicular fluids and associated with different blastocyst grades. Donors were homogeneous and followed the same standard stimulation protocol.

RESULTS: The ratio between myo-inositol and D-chiro-inositol was significantly higher in the specimens rated as good quality blastocysts, compared to those rated as poor-quality blastocysts. In this study, almost all the transferred blastocysts were graded as good quality and were correlated to lower D-chiro-inositol content in the follicular fluid; the implantation rate and pregnancy rate were satisfying. Our data suggest that the reduction of such ratio in follicular fluid seems to play a negative role in follicular development.

CONCLUSIONS: We found a correlation between myo-inositol/D-chiro-inositol ratio in follicular fluid and blastocyst quality. The value of this ratio may represent a new biomarker for estimating the good features of blastocysts, and a prognostic factor of embryo implantation and pregnancy success. Moreover, the pre-treatment with myo-inositol in women undergoing in vitro fertilization (IVF) may improve oocyte quality and ART outcome.

Clinical trial registration number: NCT03055442 (ClinicalTrials.gov registry).

Key Words:
Blastocyst quality, D-chiro-inositol, Follicular fluid, Implantation rate, intracytoplasmic sperm injection, *In vitro* fertilization, Myo-inositol, Myo-inositol/D-chiro-inositol ratio, Pregnancy rate.

Abbreviations

BMI: body mass index; DCI: D-chiro-inositol; FF: follicular fluid; FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; hCG: human chorionic gonadotropin; ICSI: intracytoplasmic sperm injection; IVF: *in vitro* fertilization; LH: luteinizing hormone; MI: myo-inositol; PCOS: polycystic ovary syndrome.

Introduction

Follicular fluid (FF) is a metabolically active microenvironment where oocytes are hosted and grow; it plays a pivotal role in the processes of fertilization and embryogenesis. Human follicles containing good quality oocytes were shown to have higher concentrations of myo-inositol (MI) in FF[1]. The positive correlation between MI concentrations and FF volume, estradiol (E2) levels and better developmental potential of the oocytes, prompted to state that higher MI levels in FF may be related to the wellbeing of follicle as well as the quality of oocytes and embryos[1]. A research study carried out in mice[2] confirmed MI key role in improving oocyte and embryo quality. MI is one of the nine stereoisomeric forms of inositol, a compound having a 6-carbon ring with a hydroxyl group attached to each carbon of the ring. The stereoisomers of inositol result from the epimerization of the six hydroxy groups. Among these isomeric forms, MI stands out for its essential biological functions. Besides MI, another notable inositol stereoisomer is D-chiro-inositol (DCI), a product obtained from MI by the epimerization of the C1 hydroxyl group. It is an enzymatic reaction catalyzed by an epimerase, which acts under in-

Corresponding Author: Giovanni Monastra, MD; e-mail: g.monastra@gmail.com

sulin control and according to the specific tissue requirements. Despite their relevant chemical similarities, MI and DCI display different biological functions. In particular, MI in the ovary is devoted to glucose uptake and FSH signaling whereas DCI mediates insulin-induced testosterone synthesis. A recent theory states that in the ovary of polycystic ovary syndrome (PCOS) hyperinsulinemic patients, there is a higher and abnormal MI to DCI epimerization. This implies DCI rise and subsequent MI deficiency[3]. Such situation was supposed to weaken FSH signaling, which ends to cause a worse oocyte quality and can induce the ovarian hyperstimulation syndrome[3]. In agreement with this theory, it was shown that hyperinsulinemia overstimulates epimerase activity in the ovary, increasing drastically MI to DCI conversion in FF of insulin resistant PCOS patients[4]. These results were confirmed by Larner et al[5] in theca cells. Moreover, it was discovered that, when DCI is administered by oral route, its FF concentration increases and such alteration worsens oocyte quality and ovarian response[6]. Having in mind the important role that follicular microenvironment exerts in the oocyte development and the different biological functions of MI and DCI, our study aimed to examine whether the ratio between the two-inositol stereoisomers in FF may correlate with blastocyst quality and may serve as a biomarker and a prognostic factor of embryo implantation and pregnancy.

Patients and Methods

Patients

This is a prospective, observational, study performed at IAKENTRO Fertility Centre (Thessaloniki, Greece) from recruitment starting date on October 1, 2016 to the completion date on November 30, 2016. The study was approved by the IAKENTRO Fertility Centre Review Board. The registration number on the ClinicalTrials.gov registry is NCT03055442. The work described here has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

The study included 8 strictly healthy egg donors, with mean age of 23.8 (SD ± 3.2) years, and mean BMI of 21.5 (SD ± 2.3) kg/m^2, participating in the donation program of IAKENTRO not for the first time, and 11 recipient couples undergoing *in vitro* fertilization (IVF). All the male partners were normospermic. The nature of the study was explained in detail to the patients. A written consent was obtained from all participants regarding the oocyte donation program.

Inclusion and Exclusion Criteria

The inclusion criteria of FF in this study were: (1) Clear follicular aspirate obtained during oocyte retrieval; (2) Each specimen of FF containing only one oocyte.

The exclusion criteria were the following: (1) Presence of hemolysis in FF.

Outcomes

The primary outcome was to assess whether different blastocyst quality may be correlated to specific MI/DCI ratios detected in the FFs. The secondary outcome was to evaluate whether such ratio can be a biomarker for the success of embryo implantation and pregnancy.

Healthy Donor Stimulation Protocol

A standard fixed 6th day antagonist protocol (Orgalutran, Organon, Roseland, NJ, USA) with 300 IU/day Menotropin FSH + LH (Merional Highly Purified HMG, Angelini Pharma Hellas s.a., Achaias, Kifisia, Greece) starting on day 2 of the cycle was used for the ovarian stimulation. An ultrasound control was performed on day 2 of the cycle to detect any ovarian cyst. In the 5th and 8th day of the stimulation, ultrasound control of the growing follicles and measurement of blood E2 levels were performed to evaluate ovarian response and manage stimulation accordingly based on E2 concentration, as well as the number and size of ovarian follicles. Daily gonadotropin-releasing hormone (GnRH) antagonist 0.25 mg was administered at the evening of day 6 of stimulation and continued up to the evening of the day prior to triggering ovulation. Final oocyte maturation was achieved by administrating either 10,000 IU of Human chorionic gonadotropin (hCG, Pregnyl, NV Organon, Roseland, NJ, USA) or 0.2 mg of GnRH agonist (Arvekap 0.1 mg, IPSEN EPE, Athens, Greece), in cases at risk of ovarian hyperstimulation, when three or more follicles, with a diameter ≥17 mm, were detected at ultrasound examination. Transvaginal oocyte aspiration was performed 36 h after triggering ovulation by ultrasound-guided follicle puncture by the same operator.

Collection of Follicular Fluid (FF) and Intracytoplasmic Sperm Injection (ICSI)

Clearly visible follicles from both ovaries of each donor were singularly aspirated and sto-

red in separate vials. Following isolation of the oocyte-cumulus complexes, if any present, the FF volume of each vial was centrifuged for 10 min at 600 g to remove debris, measured and stored at -20°C until assayed.

Isolated oocytes were collected in Quinn's Advantage Medium with buffer HEPES (SAGE, Origio, Denmark) supplemented with 10% serum substitute supplement (SSS), IRVINE (Alamo, TN, USA), covered by mineral oil (Ferticult, FertiPro NV, Beernem, Belgium). Next, the oocytes were washed and transferred in dishes covered again with human tubal fluid (HTF) and SAGE supplemented with 10% SSS, IRVINE. The dishes were kept in the incubator under the appropriate conditions (37°C temperature, 6% CO_2 and 100% humidity). 2 h later the oocytes were denudated from the cumulus cells using hyaluronidase in ferticult flushing (80 IU/ml, Fertipro, NV, Beernem, Belgium) as well as mechanical handling. Oocytes were washed two more times in HTF medium and finally were kept in the incubator in dishes covered with HTF and HEPES (SAGE) supplemented with 10% SSS IRVINE covered by mineral oil (Ferticult) until ICSI time. Then, the fertilized oocytes were transferred one by one in drops of SAGE 1step medium (Origio) covered by mineral oil (Ferticult) and kept in an incubator under appropriate conditions (37°C temperature, 6% CO_2 and 100% humidity). After 16-20 h, the oocytes were observed under the microscope and fertilization was considered successful if two pronuclei were present. The embryos remained in the same dishes (1 step medium, Origio) and were evaluated and labeled according to the morphology, under the microscope at day 2 (cleavage stage) and day 5 (blastocyst stage). The embryos were either transferred to recipients or vitrified after day 5 assessment.

Embryo Grading System

Day 5 embryos were graded according to Gardner's grading system[7]. In this study, we have grouped the samples only on the basis of "Blastocyst development and stage status" (Table I) considering the expansion and hatching stage as first priority when evaluating blastocysts as high-quality[8].

Dosage of MI and DCI in FF

Quantification of MI and DCI levels (μmol/l) was carried out at Mérieux NutriSciences Italia (Resana, Treviso, Italy) with the same procedure adopted in a previous study[4]. Briefly, after extraction with organic solvents and derivatization, sample analysis was made by gas chromatography-mass spectrometry with Agilent 6890 (Agilent, Santa Clara, CA, USA). The injection (1.0 μl) was performed in a split-less mode at 270°C, using a capillary column Agilent 122-5532 DB-5 ms (0.25 mm x 30 m x 0.25 μm). The total run-time lasted 15 min: oven at 70°C from 0 to 1 min; 20°C/min to 150°C; 10°C/min to 240°C; 4 min at 320°C post-run. The flow rate was fixed at 1.2 ml/min, and the results analyzed by a MS 5973 Network Series detector in sim mode. MI/DCI ratio was calculated as the amount of MI (μmol/l) divided by the amount of DCI (μmol/l).

Statistical Analysis

Descriptive statistics summarizing quantitative variables included median, 25th and 75th percentiles, minimum and maximum. Normality assumption of variable distribution was controlled by Shapiro-Wilk test. In consideration that these data show a non-normal distribution, comparison among the 4 grades was performed by Kruskal Wallis test, whereas Wilcoxon-Mann-Whitney test was used to compare quantitative variables between the two groups (grade 4+3 *vs.* 2+1).

Data are presented using box plot. Statistical analysis was implemented at two-sided with a 0.05 significance level, using SAS® version 9.2 (SAS Institute Inc. 100 SAS Campus Drive Cary, NC, USA) and Stata™ version 8.2 (StataCorp LLC, College Station, TX, USA).

Table I. Embryo Grading System (see Gardner et al. 2007). The blastocyst quality increases from grade 1 towards higher numbers.

Expansion grade	Blastocyst development and stage status
1	Blastocoel cavity less than half the volume of the embryo
2	Blastocoel cavity more than half the volume of the embryo
3	Full blastocyst, cavity completely filling the embryo
4	Expanded blastocyst, cavity larger than the embryo, with thinning of the shell
5	Hatching out of the shell
6	Hatched out of the shell

Results

Donors (n = 8) were homogeneous for age and BMI; the same was found for recipients (n = 11) (Table II).

From the 8 donors, we picked up 108 specimens, but we ended up analyzing 32 samples coming from all the donors; we excluded the remaining FFs because 33 of them were without oocytes, and 43 had hemolysis. In blood MI/DCI ratio is different from that of FF and for avoiding any interference that could modify the original ratio in the FF, these specimens were discarded. After dosage, 2 samples were not considered in the statistics, due to their outliers compared to those of the respective group. The collected specimens of FF were marked with the identical progressive number given to the respective oocyte. After grading embryos (day 5), the same grade was assigned to the corresponding FF sample in order to obtain a direct correlation between blastocyst quality and MI/DCI content in FF. The grade ranged from 4 to 1. MI/DCI ratios in FF specimens were compared pairwise. Only the differences between grade 4 *vs.* grade 2, and grade 4 *vs.* grade 1 were found significant by Wilcoxon-Mann-Whitney test: p=0.0433 and p=0.0198, respectively. Concerning the other groups, probably an increase in the sample number will allow to reach the statistical significance of the differences (Table III and Figure 1).

Then, the values were pooled in two groups, with grade 4+3 and grade 2+1. These two sets displayed a significant difference in MI/DCI ratio. Good quality blastocysts (i.e. grade 3 and 4) were found having such value very close to 70:1 or more, in the corresponding FF. Of note, this ratio gets about 90:1 only in FF samples correlated with grade 4 blastocysts (Table IV and Figure 2).

Unlike Chiu et al[1], no correlations were found between FF volume and its MI content (data not shown).

The results of the ICSI were not affected by sperm concentration, motility and morphology. These parameters were within the normal range, being all the male partners evaluated as normospermic. Implantation and pregnancy rates were 38.1% and 54.5%, respectively, in agreement with the normal standard of our center. No positive outcome was obtained when it was necessary to implant two blastocysts (both grade 1), with low MI/DCI ratio.

Table II. Age and BMI of donors and recipients with means ± SD.

Donors	Recip.	Donors	Recip.
Age (years)	Age (years)	BMI (kg/m^2)	BMI (kg/m^2)
21	44	22.4	22.5
25	46	17.3	32.0
24	40	21.8	27.7
30	44	22.5	19.9
26	41	24.2	23.5
22	39	18.8	19.8
21	39	23.0	19.1
21	35	21.7	19.7
	45		22.5
	43		21.3
	48		18.6
Mean 23.8	**42.18**	**21.5**	**22.42**
SD ± 3.2	**± 3.76**	**± 2.3**	**± 4.10**

Discussion

This is the first study attempting to relate the concentrations of MI and DCI in FF to the quality of blastocysts. In our study the oocytes were taken from strictly healthy young women (egg donors), subjected to ovarian stimulation, whereas the sperm for fertilization was given by the male partner of each couple undergoing IVF (noteworthy, all the men were normospermic). We found that good quality blastocysts, marked with grade 4 and 3,

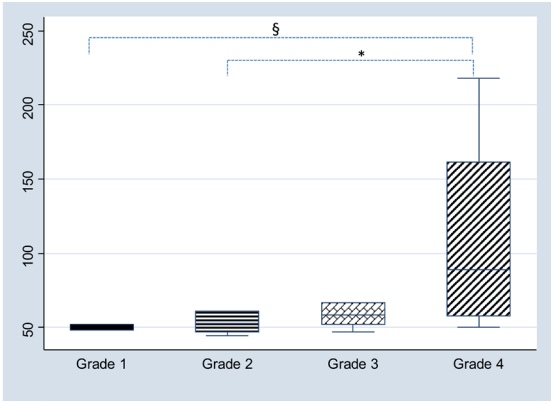

Figure 1. The blastocyst quality increases from grade 1 to grade 4. Data are presented as box plots. Kruskal Wallis test was used for the analyses. Pairwise comparison between grades was performed by Wilcoxon-Mann-Whitney test. Only Grade 4 is significantly different from the Grades 2 and 1 ($^*p$=0.0433; $^§p$=0.0198). Other statistical differences were not found. Sample size: grade 4: 14; grade 3: 7; grade 2: 6; grade 1: 3. Symbols explanation: the two "whiskers" show the minimum value and maximum value; the rectangle is given by the values between the 25th and 75th percentile; the line inside the rectangle is the median. Abbreviations: myo-inositol (MI), D-chiro-inositol (DCI).

Table III. The ratio between myo-inositol and D-chiro-inositol in follicular fluid (FF) specimens divided in groups according to the different grades (4, 3, 2 and 1 used by Gardner et al) assigned to the corresponding blastocyst at the 5th day after oocyte pick-up. The blastocyst quality increases from grade 1 to grade 4. Statistical significance: grade 4 vs. grade2 (*p=0.0433); grade 4 vs. grade 1 (§p=0.0198). Wilcoxon-Mann-Whitney test was used for the pairwise comparisons.

Variable	Group	N	MI/DCI ratio median	25th Pctl	75th Pctl	Minimum	Maximum	Kruskal Wallis test
MI/DCI ratio	grade 4	14	88.883*§	57.391	161.429	49.714	218.333	
	grade 3	7	58.421	52.143	66.190	46.765	170.000	0.0273
	grade 2	6	52.551*	46.970	60.455	44.000	144.000	
	grade 1	3	48.286§	47.917	51.667	47.917	51.667	

Abbreviations: myo-inositol (MI), D-chiro-inositol (DCI).

Table IV. The ratio between myo-inositol and D-chiro-inositol in follicular fluid (FF) specimens divided in groups according to the different scores (4, 3, 2 and 1 used by Gardner et al) assigned to the corresponding blastocyst at the 5th day after oocyte pick-up. The blastocyst quality increases from grade 1 to grade 4. Statistical significance: The samples were pooled in two groups by grade (grade: 4+3 and 2+1) and the median of their MI/DCI ratios compared. The difference was found statistically significant.

Variable	Group	N	MI/DCI ratio median	25th Pctl	75th Pctl	Minimum	Maximum	W.M. Whitney test
MI/DCI ratio	grade 4-3	21	66.190	53.824	142.000	46.765	218.333	
	grade 2-1	9	49.545	47.917	55.556	44.000	144.000	0.0099

Abbreviations: myo-inositol (MI), D-chiro-inositol (DCI).

were correlated with higher rates of MI/DCI found in FF, with satisfying results in IVF, compared to blastocysts assessed as grade 2 and 1.

We allocated our FF specimens into two groups, correlated with grade 4+3 and grade 2+1 blastocysts, respectively. Data analysis allowed us to define the good quality threshold related to the ratio between MI and DCI in FF as basically very close or higher than 70:1 (up to 100:1). The reduction of the ratio under this value was shown to exert negative consequences on blastocyst quality, assessed as grade 2 and 1. Our data suggest that a higher MI/DCI ratio in FF correlates positively with good quality blastocysts, therefore constituting a promising parameter for the success of embryo implantation and pregnancy in ICSI. We did not find correlations between FF volume (i.e. the follicular volume) and its MI content, differently from Chiu et al[1]. The explanation might be due to the different physiologic features of the FF donors: in our study, enrolled subjects were fertile healthy women, aged 23.8 ± 3.2 years, whereas in Chiu et al[1] study two groups of women, aged 33.7 ± 3.5 and 33.8 ± 4.1 years (mean and SD) with fertility problems, were included.

Several experimental and clinical data were gathered to better understand the intriguing relation between inositol and many physiological functions. Normally, MI/DCI ratio in blood is about 40:1[9], whereas in FF it is up to 100:1[4]. In general, the different distribution reflects the distinct roles that these two stereoisomers play within the

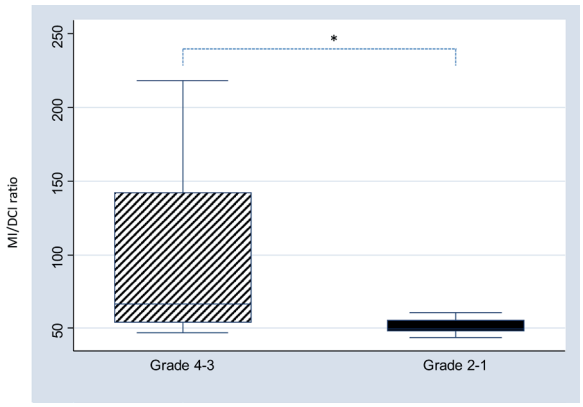

Figure 2. The blastocyst quality increases from grade 1 to grade 4. Data are presented as box plots. Wilcoxon-Mann-Whitney test was used for the analyses. The difference between the two groups (grade 4+3 *vs.* grade 2+1) was found significant (*p=0.0099). Sample size: grade 4+3: 21; grade 2+1: 9. Symbols explanation: the two "whiskers" show the minimum value and maximum value; the rectangle is given by the values between the 25th and 75th percentile; the line inside the rectangle is the median. Abbreviations: myo-inositol (MI), D-chiro-inositol (DCI).

different tissues and organs[10-12], according to the specific tissue needs[13]. This enzymatic activity is drastically reduced in many tissues (not all) when insulin resistance arises.

Inositol, as phosphatidyl-MI, is found in cell membranes; it is the precursor of inositol trisphosphate (InsP3) acting as second messenger with the role to regulate the activities of important hormones such as insulin, FSH, thyroid stimulating hormone (TSH) and serotonin[14]. In particular, considering the mechanisms of glucose metabolism regulated by insulin, it has been shown that the activation of glucose transporters and glucose utilization take place under the regulation of MI, whereas the glycogen synthesis is controlled through DCI[11,15]. On the other hand, in the ovary, FSH signaling and glucose uptake need a MI-based second messenger, while insulin-mediated androgen production requires DCI-based second messenger[16,17]. All these activities have several promising therapeutic applications[18-20].

The relevance of MI concentrations in FF has been previously documented, as a positive correlation was found by Chiu et al[1] between increased MI concentrations and better developmental potential of the oocytes. That finding prompted to state that higher levels of MI in FF may be related to the wellbeing of follicle and quality of oocytes and embryos[1]. A research study carried out in mice[2] showed that the proportion of normally fertilized oocytes with two pronuclei (2PN), the number of 2-cell embryos developed, and the percentage of normality of the post-implantation embryos were significantly higher in germinal vesicle (GV) oocytes cultured in maturation medium containing MI compared with control medium. Furthermore, a significant improvement in the development of post-implantation following the transfer of 2-cell embryos to pseudo pregnant mice was demonstrated. Noteworthy, the authors detected spontaneous intracellular Ca^{2+} oscillations within oocytes at germinal vesicle (GV) stage. Moreover, they have seen that an earlier onset of these Ca^{2+} signals was induced by MI treatment. On the bases of these data, the authors suggested that the meiotic maturation and the subsequent developmental potential of an oocyte may be increased by the direct MI availability[2]. Thus, MI can be defined as "high quality" biomarker for oocyte evaluation. It is noteworthy that Ca^{2+} has been shown to play a pivotal role in the initiation of mammalian oocyte maturation[21,22]. Increasing evidence has suggested that the phosphoinositide pathway is of prime importance in mobilizing Ca^{2+} within the cells[23,24]. Since MI is a precursor of phosphoinositide, it was postulated that MI in FF may undergo metabolism to inositol phospholipids and ultimately to Ins(1,4,5)P3 during the maturation of human oocytes[1]. A key study[4] has given a further contribution to enrich this picture, by demonstrating that hyperinsulinemia increases MI to DCI transformation in the FF of insulin resistant PCOS patients. The authors found that MI/DCI ratio was modified in PCOS women affected by hyperinsulinemia. As previously shown, MI in addition to the improvement of hormone profile and the restoration of ovulation can also normalize menstrual cycle in lean and obese PCOS patients. It is also able to reduce the rFSH units to be used in IVF, and it enhances oocyte and embryo quality[25,26]. It was also demonstrated in euglycaemic PCOS women, that the total number of oocytes retrieved did not differ in the two groups treated with MI or DCI. Contrary to DCI administration, MI showed to induce a significant growth in the number of mature oocytes and a reduction of the immature ones. In agreement with these results, increase of high quality embryos and total number of pregnancies were obtained by MI *vs.* DCI. As mentioned above, each tissue has a typical MI/DCI conversion rate depending on the specific needs[12,15]. It was demonstrated that the ratio of these two insulin mediators was itself insulin dependent. Indeed, in type 2 diabetes patients, the ratio between DCI and MI decreased with reduced synthesis of DCI, owing to a decrease of the epimerase activity[15,27]. However, ovaries and testicles never become insulin resistant[3]. Based on these data and those ones demonstrating a higher efficacy of MI *vs.* DCI in ART[25], recently a theory about the existence of a "DCI paradox" in the ovary was formulated[3]. It can be speculated that hyperinsulinemic PCOS probably have a higher stimulation of epimerase that induces an increase of MI transformation to DCI in the ovary, resulting in a decrease of MI/DCI ratio, which means a DCI overproduction and a MI deficiency at ovarian level. Such MI depletion may be accountable for the poor quality of oocytes in these women[28]. In consideration of the effect due to MI supplementation in reducing rFSH IU employed in IVF cycles, MI deficit in the ovary could also weaken FSH effects, inducing the resistance to FSH, with the consequence of adding necessarily more rFSH IU to attain a correct ovarian stimulation[29]. These findings agree with those ones obtained in another study[5], where the authors made use of theca cells from normal cycling women, with

physiological insulin sensitivity, and theca cells from PCOS women, having augmented insulin sensitivity, to measure MI/DCI ratio and MI to DCI epimerase activity. They found that in insulin-sensitive PCOS theca cells, the inositol imbalance shows a contrary direction in comparison with that detected in cells resistant to insulin. In insulin-sensitive PCOS theca cells a decreased MI/DCI ratio and an increased MI to DCI epimerase activity were observed. These data further strengthen the idea that these two parameters are strictly related with insulin resistance and sensitivity. Finally, we remind a study showing that the treatment with high doses of DCI by oral route may worsen oocyte quality and ovarian response[6]. The results demonstrated that the total rFSH units augmented significantly. The number of immature oocytes increased significantly when elevated doses of DCI were administered. Furthermore, in the group treated with DCI at the dose of 2.4 g, the number of meiosis II oocytes was significantly inferior compared to the control group. The harmful effect, due to the rise of DCI levels in the ovary, was clear, since the number of grade I embryos significantly decreased by increasing DCI supplementation.

Conclusions

The results of our study agree with previous findings mentioned above confirming the importance of the different roles exerted by MI and DCI. Indeed, MI has induced a favorable effect on all parameters analyzed, whereas DCI augmentation showed a detrimental impact. For oocyte and blastocyst evaluation, we can state that MI and DCI are, respectively, "high quality" and "low quality" biomarkers. The present study was able to identify a threshold (MI/DCI content in FF = 70:1) that correlates with blastocysts quality. Indeed, values of about 70:1 or higher correspond to good quality blastocysts fit to get a successful IVF. This finding suggests that FF MI/DCI ratio might serve as a promising functional indicator. Our data display a new advancement in the understanding of very complex physiological processes related to women fertility, offering focused strategies to increase the chances of getting pregnant, even though there are some limitations to the study, such as the small sample size.

However, these findings open new perspectives of research. Among them, it would be interesting to correlate MI/DCI ratio in FFs with oocyte quality as well as detect MI/DCI ratio in FFs lacking oocytes. Finally, from the therapeutic point of view, on the base of our study, we suggest that the pre-treatment with MI in women undergoing IVF improves oocyte quality and ART outcome, providing a focused intervention in this area.

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Conflict of interest

The authors declare no conflicts of interest.

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