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Effects of Myo-Inositol supplementation on oocyte's quality in PCOS patients: a double blind trial

L. CIOTTA, M. STRACQUADANIO, I. PAGANO, A. CARBONARO, M. PALUMBO, F. GULINO

Microbiological and Gynecological Science Department, Gynecology Section "Santo Bambino" Hospital (Catania), University of Catania (Italy)

Abstract. – *Background:* **Polycystic ovary syndrome is the most common cause of chronic anovulation infertility in women in fertile period, and it's characterized by an increased production of androgens and estrogens. The administration of D-chiro-inositol, a B complex vitamin, was associated with a decreased of serum testosterone and simultaneously, due to its ability to increase insulin sensitivity, women who received D-chiro-inositol showed a great improvement of the ovulary function. Besides, the supplementation of inositol improves the oocytes' quality and increase the number of oocytes collected after ovarian stimulation in patients undergoing IVF (*in vitro* fertilization).**

Aim: **The aim of our study is to determine the effects of myo-inositol on oocyte's quality on a sample of women with polycystic ovary syndrome.**

Material and Methods: **The patients were divided into two groups: patients of Group A intook 2 g of myo-inositol + 200 µg of folic acid (Inofolic®, LO.LI. Pharma, Rome, Italy) 2 times a day, continuously for 3 months, while Group B only 200 µg of folic acid, both groups took the treatment twice a day, continuously for 3 months.**

Results: **At the end of treatment, the number of follicles of diameter >15 mm, visible at ul-trasound during stimulation, and the number of oocytes recovered at the time of pick-ups were found to be significantly greater in the group treated with myo-inositol, so as the aver-age number of embryos transferred and embryo Score S1. Significantly reduced was the av-erage number of immature oocytes (vesicles germ and degenerated oocytes) too.**

Conclusions: **These data suggest that myo-inositol may be useful in the treatment of PCOS patients undergoing ovulation induction, both for its insulin-sensitizing activity, and its role in oocyte maturation.**

Key Words:

Inositol, Oocyte's quality, Polycystic ovary syndrome, Infertility, *In vitro* fertilization.

Introduction

Polycystic Ovary Syndrome (PCOS) is a complex disease characterized by various endocrine disorders that can be the potential cause of anovulation and hyperandrogenism condition.

This heterogenous syndrome affects about 5-10% of female population in reproductive age, and it can be considered as the most common endocrine disorder affecting women during reproductive life[1].

PCOS cannot be merely considered a local ovarian dysfunction or a central hypothalamus-ovary-pituitary defect, but it is the expression of a complex functional alteration of the whole reproductive system.

Under a hormonal point of view, the micropolycystic ovary is characterized by an increased production of androgens and estrogens, and a dissociation of gonadotropins serum concentrations: elevated luteinizing hormone (LH), low or normal follicle stimulating hormone (FSH) and LH/FSH ratio that usually exceeds 2.5 in the typical forms.

In the blood of PCOS patients testosterone (T), androstenedione (AS), dehydroepiandrosterone (DHEA), DHEA-S (sulfate), 17-hydroxyprogesterone (12-OHP) and estrone resulted elevated. The circulating levels of sex hormone-binding globulin (SHBG) are instead lower.

The enhancing peripheral conversion of androstenedione to estrone leads to the modest relative hyperandrogenism.

SHBG levels are reduced of about 50% due to the increased levels of testosterone[2-4].

The syndrome's etiology is still unknown, but it is probably multifactorial, due to an excessive E_1 production, or to an alteration of the primitive hypothalamic regulation and of the ovarian and/or adrenal steroidogenesis.

Corresponding Author: Mariagrazia Stracquadanio, MD; e-mail: mariagrazia.stracquadanio@gmail.com

The diagnosis of PCOS is based on the clinical, hormonal and ultrasound patterns. In accordance with Rotterdam Criteria, drawn in 2003, PCOS diagnosis can be made only after the exclusion of other causes of hyperandrogenism and amenorrhea, and in the presence of at least 2 of the following criteria:

- Oligo- and/or anovulation with menstrual irregularities;
- Elevated levels of circulating androgens or clinical manifestation of hyperandrogenism;
- Transvaginal pelvic ultrasound evidence of micropolycystic ovary.

Due to the pulsatility of LH, only one blood parameter is not enough for the PCOS diagnosis, and there is no unanimous consensus on which androgen blood's level should be considered for a precise diagnosis (total or free testosterone, testosterone/SHBG ratio or androstenedione).

Usually, elevated levels of only DHEA or 17-OHP may exclude the diagnosis of PCOS.

Since menarche, or after a short period, menstrual cycles show an irregular rhythm. In many cases they gradually distance themselves from each other, up to result in short periods of amenorrhea or in permanent amenorrhea. Menstrual dysfunction in women affected by PCOS may manifest in different ways, but the probably most common way is anovulation with erratic bleedings.

Androgens excess is responsible for hirsutism, oily skin, acne and, in the ovary, for the thickening of the tunica albuginea. The degree of hirsutism can be measured with the Ferriman-Gallwey score.

In rare cases virilization patterns can be observed, with increased size of clitoris, muscle mass hypertrophy, deep voice, temporal balding and masculine aspect. In these cases, however, a lower ovarian or an adrenal androgen-secreting neoplasia must be excluded.

At the same time an overweight pattern, up to obesity can be associated to the syndrome.

PCOS is one of the most common endocrine causes of female infertility: if you want to get pregnant, ovulation should be induced[5].

Ovulatory cycles are obtained, usually, after the overweight correction, or immediately after the estrogen-progestins suspension. If it does not happen, ovulation should be induced pharmacologically (usually associated with metformin administration)[6].

Clomiphene is a drug normally used for this purpose: it is a weak estrogen that acts also as anti-estrogen. Probably, it interacts with the hypothalamic estrogen receptors, displacing the endogenous estradiol and creating a condition of artificial hypoestrogenism, due to its biological activity almost absent in this district. Hypothalamic centers, responsible for gonadotropin-releasing hormone (GnRH) release are thus stimulated to greater activity. Following the administration of clomiphene, in fact, the frequency of pulsatile secretion of LH and FSH increases, while the amplitude remains unchanged. Ovulation in PCOS is induced in 80% of cases, while pregnancy occurs in 20% of cases.

Where no response to the treatment with clomiphene and metformin was obtained, or where an *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) was necessary, ovulation induction was performed by the administration of gonadotropins. Gonadotropins used for this purpose are obtained from the urine of postmenopausal women (Menotrophin). Recently, gonadotropins obtained with biosynthetic technique from recombinant DNA have been introduced (Follicotropin α and Follicotropin β). The goal of the therapy with gonadotropins, or rather with FSH, is acting on the follicles in the last stage of their maturation process that, under physiological conditions is restricted to the first two weeks of the menstrual cycle in which ovulation occurs[5,7].

The aim of our study is to determine the effects of Myo-Inositol, a compound belonging to vitamin B complex, on oocyte's quality in a group of patients with PCOS, suffering from chronic anovulation and infertility, undergoing medically assisted reproduction techniques (ART), such as IVF and ICSI.

Scientific studies have shown that D-chiro-inositol, thanks to its ability to increase insulin sensitivity, has beneficial effects on ovulation and on the androgens production in women with PCOS. The administration of D-chiro-inositol was associated with a decreased of serum testosterone[8] and increased of SHBG concentration. Simultaneously to the reduction of insulin secretion, women who received D-chiro-inositol showed a great improvement of the ovulary function[9,10]. Scientific evidence has also shown that the supplementation with inositol contributes to reducing the amount of FSH necessary to ovulation, to improving oocyte's quality (reduction of the total amount of the germinal vesicles and the degener-

ated oocytes) and to increasing the number of oocytes collected after ovarian stimulation in patients undergoing ART techniques, as IVF or ICSI[11-13]. Inositol cannot be defined exactly as a vitamin, but it is considered a vitamin factor belonging to B complex. In the human organism it is present in the phospholipids, and it can stimulate endogenous production of lecithin. Its role also includes a specific biological activity of control on fat and sugar metabolism, and on the cellular function of the nervous system. It is also essential to hair growth and it can prevent baldness. Scientific studies revealed that diabetic subjects eliminate amounts of inositol significantly higher that no-diabetic ones[14].

In case of insulin resistance or type II diabetes, inositol helps to improve the whole clinical pattern. In these cases, inositol may be useful to prevent and to correct pathophysiological mechanisms underlying the metabolic and reproductive abnormalities related to PCOS[15-18].

Materials and Methods

All the patients were enrolled and treated in the Department of Gynecological Sciences ("Santo Bambino" Hospital, Catania), at the Gynecological Endocrinology Clinics and Human Reproduction Pathophysiology Centre. In the 12-month enrollment phase a total of 34 women, aged <40 years with polycystic ovary syndrome (PCOS) were selected.

PCOS diagnosis was indicated by oligo-amenorrhea (six or fewer menstrual cycles during a period of one year), hyperandrogenism (hirsutism, acne or alopecia) or hyperandrogenemia (elevated levels of total or free testosterone), and typical feature of ovaries at ultrasound scan.

Concomitant endocrine and metabolic pathologies, as hypothyroidism, hyperthyroidism, diabetes mellitus, androgen-secreting cancers, adrenal hyperplasia, Cushing syndrome were excluded.

The ICSI or IVF procedures were recommended after the evaluation of the sperm semen of the male partner.

According to a randomization table, patients were divided into two groups: patients of Group A intook 2 g of myo-inositol + 200 µg of folic acid (Inofolic®, LO.LI. Pharma, Rome, Italy) 2 times a day, continuously for 3 months, while Group B only 200 µg of folic acid, both groups took the treatment twice a day, continuously for 3 months.

Oocyte's quality assessment was performed after the oocyte pick-up conducted during the assisted reproductive technology procedure in which patients have been submitted. The ICSI or IVF includes several phases (ovarian stimulation, oocyte collection, oocyte quality assessment, oocytes in-vitro fertilization, embryo culture and scoring, embryo transfer), all crucial for the success of the technique. They were all followed by the medical team of the Reproduction Pathophysiology Centre of "Santo Bambino" Hospital, in Catania at the IVF clinic with the attached surgery room.

Statistical Analysis

The comparison between Group A and Group B was performed using:

- X^2 test for qualitative data (β-hCG positivity);
- Student *t* test for quantitative data normally distributed (age, BMI, total FSH units administered, number of follicles of diameter >15 mm);
- U test for quantitative data not normally distributed (days of stimulation, E_2 maximum peak, number of oocytes retrieved).

Results

During the study period, patients were randomly divided into two groups, as described before, and the study was performed in a double-blind design.

No significant differences were found between the two groups in mean age and body mass index (BMI).

Total r-FSH units administered for the ovarian stimulation were significantly reduced in group A.

As reported in literature, peak E_2 levels at human chorionic gonadotropin (hCG) administration were lower in group A, but our data were not statistically significant.

Two cycles were cancelled in group A, whereas in group B five cycles were suspended, because of peak E_2 >4,000 pg/mL (risk of hyperstimulation).

The number of follicles with a diameter >15 mm, visible at ultrasound scan during stimulation, and the number of oocytes retrieved at the pick-up resulted significantly higher in the myo-inositol-treated group (Table I).

Table I. Retrieved oocytes at the pick-up.

Group	Median	Percentiles 25°	Percentiles 75°
A	12	10	16
B	8.50	6.25	10.75
	$P < 0.05$		

The mean number of immature oocytes (germinal vesicles and degenerated oocytes) was significantly reduced, and there was an increasing trend of the rate of oocytes in metaphase II (MII), that are oocytes characterized by not visible germinal vesicles and visible first polar body (Table II).

No statistical significance in the number of fertilized embryos was emerged, but in group A the mean number of transferred embryos resulted significantly higher, with higher amounts of score 1 embryos in comparison with lower-quality embryos (Table III).

In compliance with the italian ART law, no more than three embryos were transferred. No differences in the total number of biochemical pregnancies were detected.

Discussion

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders. Insulin-resistance and hyperinsulinemia are strictly correlated with the phenotype of a large part of PCOS women.

A defect in the insulin's action has been suspected, probably because of a deficiency of D-chiro-inositol, that is a component of inositol phosphoglycans. Insulin-lowering drugs, particularly different forms of inositol, represent novel therapies in spontaneous ovulation restoration, with a potential positive action even on meiotic oocyte maturation. These therapies seem to directly influence steroidogenesis, by reducing androgen production in theca cells. In fact, the administration of D-chiro-inositol has been demonstrated to increase the insulin action in PCOS patients, improving ovulatory function[10], and reducing serum testosterone concentration[8, 9,19].

Nowadays, there are few data on the action and effects of myo-inositol, a precursor of D-chiro-inositol, on the anovulatory women in reproductive age or on the spontaneous ovulation in stimulated cycles.

Anyway, myo-inositol is an important constituent of the follicular microenvironment, playing a key role in the nuclear and cytoplasmic oocyte's development.

In the assisted reproduction techniques, in fact, the supplementation with myo-inositol is positively related to meiotic progression of mouse germinal vesicle oocytes, enhancing intracellular Ca^{2+} oscillation[20]. Furthermore, higher concentrations of myo-inositol in human follicular fluid provide a marker of good-quality oocytes[21].

Our study is one of the fews focusing on this molecule, that belongs to vitamin B complex, and on its effects in PCOS patients undergoing ovulation induction. Our preliminary data in our hands show that, in PCOS patients the treatment with myo-inositol and folic acid, compared with folic acid alone, reduces the number of germinal vesicles and degenerated oocytes, without compromising the total number of retrieved oocytes. These results, as other trials', suggest that myo-inositol has a positive effect on mature oocytes development[22].

Furthermore, it is well known that ovulation induction in PCOS patients is a pivotal matter, even because of the risk of the ovarian hyperstimulation syndrome[23-24]. Elevated basal serum levels of androgens are involved in the produc-

Table II. Metaphase II (MII) oocytes, degenerated oocytes and germinal vesicles.

	Group A		Group B		
	Frequency	%	Frequency	%	*P*
MII oocytes	176	82.24	160	66.87	NS
Degenerated oocytes	2	0.93	23	14.37	0.02
Germinal vesicles	3	1.4	15	9.37	0.02

Table III. Number of score 1 embryos.

	Group A		Group B		
	Frequency	%	Frequency	%	*P*
Score 1 embryos	30	68.1	9	29	< 0.01

tion of high serum E_2 levels, as typically detected in PCOS patients undergoing ovulation induction with exogenous gonadotropins.

Because myo-inositol is a D-chiro-inositol precursor, an insulin-sensitizing action on the ovary may be similarly hypothesized, with a subsequent positive action on the hormonal profile, particularly on basal serum testosterone reduction[8,9,25]. In fact, in patients treated with myo-inositol plus exogenous gonadotropins a significant reduction in E_2 levels at hCG administration was found. As consequence, it can be supposed that this protocol could be adopted to reduce the risk of hyperstimulation in such patients.

In conclusion, these observations suggest that myo-inositol may be useful in the treatment of PCOS patients undergoing ovulation induction, both for its insulin-sensitizing activity, and its role in oocyte maturation.

References

1) Sattar N, Hopkinson Z, Greer IA. Insulin-sensiting agents in polycystic ovary sin-drome. Lancet 1998; 351: 305.
2) Cianci S, et al. Rivisitando la policistosi ovarica. Aggiornamenti in ostetricia e Gine-cologia. CIC Edizioni Internazionali, 1988.
3) Flamigni C, Venturoli S, Porcu E. La sindrome dell'ovaio micropolicistico; Conside-razioni cliniche ed eziopatogenetiche. In: Genazzani, Volpe: Endocrinologia Ginecologica. Fisiopatologia, Clinica e Strategie Terapeutiche. Monduzzi Editore, 1984.
4) Filicori M, Flamigni C, Campaniello E, Meriggiola MC, Michelacci L, Valdiserri A, Ferrari P. Polycystic ovary syndrome: abnormalities and management with pulsatile go-nadotropin-releasing hormone and gonadotropin-releasing hormone analogs. Am J Obstet Gynecol 1990; 163: 1737-1742.
5) Arosio M, Persani L, Faglia G. Sindrome dell'ovaio policistico; Malattie endocrine dell'ovaio. In: Faglia G, Beck-Peccoz P. Malattie del sistema endocrino e del metabolismo. McGraw-Hill, 2006.
6) ACOG Committee on Practice Bulletins. Polycystic Ovary syndrome. Int J Gynecol Obstet 2003; 80: 335-348.
7) Toscano V. Sindrome dell'ovaio policistico. In: Andreani D, Tamburano G. Manuale di Terapia delle Malattie Endocrine e Metaboliche. Delfino Antonio Editore, 2000.
8) Costantino D, Minozzi G, Minozzi F, Guaraldi C. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. Eur Rev Med Pharmacol Sci 2009; 13 (2): 105-110.
9) Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of D chiro-inositol in the polycystic ovary syndrome. N Engl J Med 1999; 340: 1314-1320.
10) Gerli S, Papaleo E, Ferrari A, Di Renzo GC. Randomized, double blind placebo-controlled trial: effects of Myo-inositol on ovarian function and metabolic factors in women with PCOS. Eur Rev Med Pharmacol Sci 2007; 11 (5): 347-354.
11) Papaleo E, Unfer V, Baillargeon JP , Fusi F, Occhi F, De Santis L. Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. Fertil Steril 2009; 91: 1750-1754.
12) Papaleo E, Unfer V, Baillargeon JP, De Santis L, Fusi F, Brigante C, Marelli G, Cino I, Redaelli A, Ferrari A. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. Gynecol Endocrinol 2007; 23: 700-703.
13) Chiu TT, Rogers MS, Law EL, Briton-Jones CM, Cheung LP, Haines CJ. Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. Hum Reprod 2002; 17: 1591-1596.
14) Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. New Engl J Med 2002; 346: 393-403.
15) Lowenstein EJ. Diagnosis and management of the dermatologic manifestations of the polycystic ovary syndrome. Dermatol Ther 2006; 19: 210-223.
16) Azziz R, Sanchez LA, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, Taylor K, Boots LR. Androgen excess in women: Experience with over 1000 consecutive patients. J Clin Endocrinol Metab 2004; 89: 453-462.

17) American Diabetes Association. Position statement: Gestational diabetes mellitus. Diabetes Care 2004; 7(Supp. 1): S88-S90.

18) Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. The sixth report of the Joint National Committee on Prevention, Detec-tion, Evaluation, and Treatment of High Blood Pressure. Arch Int Med 1997; 157: 2413-2446

19) Baillargeon JP, Diamanti-Kandarakis E, Ostlund RE Jr, Apridonidze T, Iuorno MJ, Nestler JE. Altered D-chiro-inositol urinary clearance in women with polycystic ovary syn-drome. Diabetes Care 2006; 29: 300-305.

20) Chiu TT, Rogers MS, Briton Jones CM, Haines C. Effect of myo-inositol on the in-vitro maturation and subsequent development of mouse oocytes. Hum Reprod 2003; 18: 408-416.

21) Chiu TT, Rogers MS, Law EL, Briton-Jones CM, Cheung LP, Haines CJ. Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. Hum Reprod 2002; 17: 1591-1596.

22) Goud PT, Goud AP, Van Oostveldt P, Dhont M. Presence and dynamic redistribution of type I inositol 1,4,5-trisphosphate receptors in human oocytes and embryos during in-vitro maturation, fertilization and early cleavage divisions. Mol Hum Reprod 1999; 5: 441-451.

23) Tummon I, Gavrilova-Jordan L, Allemand MC, Session D. Polycistic ovaries and ovarian hyperstimulation syndrome: a systematic review. Acta Obstet Gynecol Scand 2005; 84: 611-616.

24) Battaglia C, Mancini F, Persico N, Zaccaria V, de Aloysio D. Ultrasound evaluation of PCO, PCOS and OHSS. Reprod Biomed Online 2004; 9: 614-619.

25) Iuorno MJ, Jakubowicz DJ, Baillargeon JP, Dillon P, Gunn RD, Allan G, Nestler JE. Effects of d-chiro-inositol in lean women with the polycystic ovary syndrome. Endocr Pract 2002; 8: 417-423.