Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial

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Objective: To determine the effects of *myo*-inositol on oocyte quality in polycystic ovary syndrome (PCOS) patients undergoing intracytoplasmic sperm injection (ICSI) cycles.
Design: A prospective, controlled, randomized trial.
Setting: Assisted reproduction centers.
Patient(s): Sixty infertile PCO patients undergoing ovulation induction for ICSI.
Intervention(s): All participants underwent standard long protocol. Starting on the day of GnRH administration, 30 participants received *myo*-inositol combined with folic acid (Inofolic) 2 g twice a day and 30 control women received folic acid alone, administrated continuously.
Main Outcome Measure(s): Primary end points were number of morphologically mature oocytes retrieved, embryo quality, and pregnancy and implantation rates. Secondary end points were total number of days of FSH stimulation, total dose of gonadotropin administered, E_2 level on the day of hCG administration, fertilization rate per number of retrieved oocytes, embryo cleavage rate, live birth and miscarriage rates, cancellation rate, and incidence of moderate or severe ovarian hyperstimulation syndrome.
Result(s): Total r-FSH units (1,958 ± 695 vs. 2,383 ± 578) and number of days of stimulation (11.4 ± 0.9 vs. 12.4 ± 1.4) were significantly reduced in the *myo*-inositol group. Furthermore, peak E_2 levels (2,232 ± 510 vs. 2,713 ± 595 pg/mL) at hCG administration were significantly lower in patients receiving *myo*-inositol. The mean number of oocytes retrieved did not differ in the two groups, whereas in the group cotreated with *myo*-inositol the mean number of germinal vesicles and degenerated oocytes was significantly reduced (1.0 ± 0.9 vs. 1.6 ± 1.0), with a trend for increased percentage of oocytes in metaphase II (0.82 ± 0.11% vs. 0.75 ± 0.15%).
Conclusion(s): These data show that in patients with PCOS, treatment with *myo*-inositol and folic acid, but not folic acid alone, reduces germinal vesicles and degenerated oocytes at ovum pick-up without compromising total number of retrieved oocytes. This approach, reducing E_2 levels at hGC administration, could be adopted to decrease the risk of hyperstimulation in such patients. (Fertil Steril® 2009;91:1750–4. ©2009 by American Society for Reproductive Medicine.)

Key Words: *Myo*-inositol, oocyte quality, ovarian stimulation, ICSI cycles

Polycystic ovary syndrome (PCOS) is a medical condition that causes irregular menstrual cycles, chronic anovulation most often manifested as oligoamenorrhea, and androgen excess, with typical ovarian ultrasound features (1). It is the most common cause of ovulatory disorders and female infertility and affects approximately 6%–10% of women of childbearing age (2). However, its pathogenesis is poorly understood.

Many investigators have focused both on impaired glucose tolerance, which affects 30%–40% of patients with PCOS (3), and on insulin resistance, which is present in a significant proportion of women with PCOS. Insulin plays a direct role in the pathogenesis of hyperandrogenemia in PCOS, acting synergistically with LH to enhance androgen production in theca cells (4). Since the report by Burghen et al. (5) in 1980, where PCOS was found to be associated with hyperinsulinemia, it has become clear that this syndrome has major metabolic as well as reproductive morbidities. The recognition of this association has also instigated extensive investigation on the relationship between insulin and gonadal function. Accordingly, this association has led to the treatment of PCOS women with insulin-sensitizing agents such as troglitazone (9), inositol (8, 10–11), and mainly metformin (12–15) for restoring spontaneous ovulation. Efficacy of metformin is still debated, both alone or in association with clomiphene citrate. Furthermore, metformin treatment is associated with a higher incidence of side events, such as nausea or vomiting and other gastrointestinal disturbance (15).

A number of small randomized and nonrandomized cohort studies have shown that women with PCOS respond to D-*chiro*-inositol (DCI) therapy, increasing ovarian activity

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and menstrual frequency (8,10). In fact, an inositol phosphoglycan (IPG) molecule containing DCI is known to have a role in activating enzymes that control glucose metabolism (6), acting as postreceptor mediator or as a second messenger of insulin signal. A defect in tissue availability or use of DCI or (IPG mediators may contribute to insulin resistance (7–8). However, the relationships among treatment outcome, anthropometric changes, and glycemic, metabolic, and lipid profile adjustments were less comprehensively studied and remain disputed. Some of the differences among the results already published may derive from differences in patient selection, because patient profiles can differ between infertility and endocrinology clinics and perhaps also in racial and socioeconomic makeup.

Only a minority of the studies where inositol was used are double-blind placebo-controlled trials, with the majority being small cohort studies. In particular, direct assessment of follicular development, ovulation, and E_2 and P blood levels has been far from comprehensive. The latter point is relevant because in women with PCOS many ovulations are accompanied by elevated E_2 and subnormal P concentrations, which may indicate a suboptimal follicular maturation and ovulation with a collection of high numbers of germinal vesicles and degenerated oocytes at ovum pick-up.

Myo-inositol (MI) and DCI are isoforms of inositol and belong to the vitamin B complex. *Myo*-inositol is widely distributed in nature, whereas DCI, the product of epimerization of C_1 hydroxyl group of MI, is relatively rare (16).

In IVF techniques, it was demonstrated that supplementation with *myo*-inositol is positively related to meiotic progression of mouse germinal vesicle oocytes, enhancing intracellular Ca^{2+} oscillation (17). Indeed, in human follicular fluids, higher concentrations of MI provide a marker of good-quality oocytes (18).

The aim of the present study was to investigate the effects of *myo*-inositol on ovarian function in women with PCOS undergoing ovulation induction for intracytoplasmic sperm injection (ICSI), treated within a randomized placebo-controlled trial.

MATERIALS AND METHODS

Patients

All patients treated in our IVF department for a period of more than 12 months were asked to participate to the study. A total of 60 women aged <40 years with polycystic ovary syndrome, indicated by oligoamenorrhea (six or fewer menstrual cycles during a period of 1 year), hyperandrogenism (hirsutism, acne, or alopecia), or hyperandrogenemia (elevated levels of total or free T) and typical features of ovaries on ultrasound scan, were enrolled in the study. Other medical conditions causing ovulatory disorders, such as hyperinsulinemia, hyperprolactinemia, or hypothyroidism, or androgen excess, such as adrenal hyperplasia or Cushing syndrome, were excluded.

The ICSI procedure was indicated after evaluation of two different sperm semen samples of the male partner. According to a randomization table, patients were assigned to receive either 2 g *myo*-inositol twice a day combined with 400 μg folic acid (Inofolic, Lo.Li. Pharma, Rome, Italy), or 400 μg folic acid only, administrated continuously. The Institutional Review Board approved the protocol, and all patients gave a written informed consent before entering in the study.

Controlled Ovarian Hyperstimulation

All patients underwent pituitary desensitization by SC administration of a GnRH agonist (Decapeptyl; Ipsen, Paris, France) from midluteal phase until the IM administration of 10,000 IU hCG. Then, controlled ovarian hyperstimulation was performed in all patients by administration of recombinant FSH (Gonal-F; Merck-Serono, Geneva, Switzerland). Starting dose was 150 IU per day. Patients were monitored by measuring the plasma concentration of 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 IM 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. Cycle was canceled if E_2 level was >4,000 pg/mL, because of high risk for ovarian hyperstimulation syndrome.

ICSI Procedure

For all patients enrolled after March 10, 2004, according to Italian IVF law, a maximum of three oocytes per patient were injected and spare mature oocytes were cryopreserved according to protocols described in previous studies (19). Oocyte and sperm preparation for conventional ICSI procedure have been thoroughly described elsewhere (20). With respect to ICSI, briefly, 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 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 μm 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 (21).

Luteal Phase

Intramuscular administration of 50 mg daily progesterone-in-oil was started on the day of ovum pick-up, and treatment was continued 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 pregnancy. 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 was used for data analysis. Baseline characteristics and ovulation induction (Table 1) were analyzed using the unpaired Student *t* test. Ovum pick-up and injection outcomes were analyzed using Wilcoxon test; pregnancy rate was compared using χ^2 analysis of Fisher exact test. Results with $P<.05$ were considered to be significant.

RESULTS

During the study period, 60 patients conforming to the inclusion criteria were randomized into two groups as described. Group A (*myo*-inositol plus folic acid) consisted of 30 patients and group B (folic acid alone) consisted of 30 control subjects. No differences were found between the two groups in mean age, body mass index (BMI), and duration of infertility (Table 1). The causes of infertility also didn't differ after randomization between the two groups.

Total r-FSH units (1,958 ± 695 vs. 2,383 ± 578; $P=.01$) and number of days of stimulation (11.4 ± 0.9 vs. 12.4 ± 1.4; $P=.01$) were significantly reduced in group A. Furthermore, peak E_2 levels (2,232 ± 510 vs. 2,713 ± 595 pg/mL; $P=.02$) at hCG administration were significantly lower in the *myo*-inositol–treated group. One cycle was canceled in group A, whereas in group B three cycles were suspended, because of E_2 peak >4,000 pg/mL.

The mean number of oocytes retrieved did not differ between the two groups, whereas in the group cotreated with *myo*-inositol the mean number of immature oocytes (germinal vesicles) and degenerated oocytes not suitable for injection was significantly reduced (1.0 ± 0.9 vs. 1.6 ± 1.0; $P=.01$), with a trend for increased percentage of MII oocytes (0.82 ± 0.11% vs. 0.75 ± 0.15%; $P=.06$; Table 2). In compliance with Italian IVF law, no more than three oocytes per patient were injected. No differences emerged in fertilization and cleavage rates, mean number of transfered embryos, and mean number of top-quality transfered embryos. A total of 21 pregnancies were obtained (11 in group A and 10 in group B; $P=.74$ by chi-square test; Table 3).

DISCUSSION

Polycystic ovary syndrome is one of the most common endocrine disorders affecting women. Insulin resistance and hyperinsulinemia are strictly inherent to the phenotype of a high proportion of women with PCOS. A defect in insulin action has been suspected, possibly as a consequence of a deficiency of DCI, which is a component of inositol phosphoglycans.

Insulin-lowering medications, particularly different isoforms of inositol, represent novel therapies for restoring spontaneous ovulation, with a potential positive effect also on human oocyte meiotic maturation. These therapies appear to influence steroidogenesis directly, reducing the androgen production in theca cells. In fact, it was demonstrated that DCI administration increases the action of insulin in patients with PCOS, thereby improving ovulatory function and decreasing serum T concentration (7, 8, 10, 11).

Actually no data exist on action and effects of MI, a precursor of DCI, on anovulatory women of reproductive age or on spontaneous ovulation in stimulated cycles.

TABLE 1

Characteristics and outcome of patients who received *myo*-inositol plus folic acid (group A; n = 30) or folic acid alone (group B; n = 30).

Variable	Group A	Group B	*P* value
No. of patients	30	30	—
Age (yrs)	36.2 ± 2.4	35.4 ± 2.5	NS
Duration of infertility (months)	46.1 ± 18.5	37.7 ± 9.6	NS
Body mass index (kg/m^2)	26.7 ± 7.5	26.3 ± 6.8	NS
PRL (ng/mL)	17.8 ± 1.9	19.1 ± 2.1	NS
TSH (mIU/L)	1.56 ± 0.95	1.66 ± 1.01	NS
Duration of stimulation (days)	11.3 ± 0.9	12.3 ± 1.4	.002
No. of 75-IU ampules or vials of FSH	26 ± 7.7	31,7 ± 9.2	.016
17β-E_2 level on day of hCG administration (pg/mL)	2,232.1 ± 510	2,713.3 ± 595	.002
No. of canceled cycles (E_2 level >4,000 pg/mL)	1	3	.003

Note: Values are mean ± SD. NS = not significant.

Papaleo. MI, PCO, and oocyte quality in ICSI cycles. Fertil Steril 2009.

TABLE 2

Oocyte maturity and embryo score in patients who received *myo*-inositol plus folic acid (group A; n = 30) or folic acid alone (group B; n = 30).

Characteristic	Group A	Group B	*P* value
No. of retrieved oocytes	8.76 ± 4.12	9.37 ± 3.31	NS
No. of MII oocytes	7.14 ± 3.49	7.07 ± 3.04	NS
MII/total oocytes retrieved (%)	0.82 ± 0.11	0.75 ± 0.15	NS (.06)
No. of mmature oocytes (GV-DEG)	1.03 ± 0.87	1.63 ± 1.01	.02
Fertilization rate	0.79 ± 0,19	0.74 ± 0,18	NS
Cleavage rate	0.88 ± 0.07	0.87 ± 0.1	NS
No. of embryos transfered	2.07 ± 0.75	1.86 ± 0.85	NS
Embryo score grade 1 (%)	0.86 ± 0.83	0.81 ± 0.83	NS
Embryo score grade 2 (%)	0.93 ± 0.80	0.74 ± 0.66	NS
Embryo score grade 3 (%)	0.31 ± 0.54	0.30 ± 0.47	NS

Note: Values are mean ± SD. The embryos were scored according to the criteria established by Veeck (26). DEG = degenerated occytes; MII = metaphase II; NS = not significant; GV = germinal vesicle.

Papaleo. MI, PCO, and oocyte quality in ICSI cycles. Fertil Steril 2009.

However *myo*-inositol is an important constituent of follicular microenvironment, playing a determinant role in both nuclear and cytoplasmic oocyte development. In fact, in IVF techniques supplementation by *myo*-inositol is positively related to meiotic progression of mouse germinal vesicle oocytes, enhancing intracellular Ca^{2+} oscillation (18). Indeed, in human follicular fluids, higher concentrations of MI provide a marker of good-quality oocytes (17).

The present study is the first trial focusing on this molecule, belonging to the vitamin B complex, and its effects on patients with PCOS undergoing ovulation induction. The preliminary data show that in patients with PCOS the treatment with *myo*-inositol and folic acid, compared with folic acid alone, reduced germinal vesicles and degenerated oocytes at ovum pick-up without compromising total number of retrieved oocytes. These results are in line with other studies, suggesting the positive effect that *myo*-inositol plays on developmental competence of maturing oocytes (24).

Furthermore, it is well known that ovulation induction in PCOS patients is a complex issue, owing to increased risk of hyperstimulation syndrome (22, 23). Therefore, elevated basal levels of serum ovarian androgens are implicated in production of elevated serum E_2 levels, typical of PCOS patients undergoing ovulation induction with exogenous gonadotropin. Because *myo*-inositol is the precursor of DCI, an ovarian insulin-sensitizing action can be similarly hypothesized with a consequently positive action on hormonal profile, particularly on reduction of basal serum T, as described elsewhere (8, 10, 25).

In fact, in gonadotropin– plus *myo*-inositol–treated patients, a significant reduction in E_2 levels at hGC administration was found, and as a consequence we suppose this approach could be adopted to decrease the risk of hyperstimulation in such patients.

In conclusion, these observations suggest that MI may prove useful in the treatment of PCOS patients undergoing ovulation induction, both for its insulin-lowering activity and its intracellular role in oocyte maturation.

TABLE 3

Pregnancy outcome of patients who received *myo*-inositol plus folic acid (group A; n = 30) or folic acid alone (group B; n = 30).

Variable	Group A	Group B	*P* value
No. of pregnancies	11	10	NS
No. biochemical pregnancies, (%)	1 (9.1)	1 (10)	NS
Implantation rate,[a] %	14.6	12.9	NS
No. clinical pregnancies, (%)	8 (26.6)	7 (23.3)	NS
No. spontaneous abortions,[b] (%)	2 (25.0)	2 (28.5)	NS

Note: NS = not significant.
[a] Total number of embryos transfered.
[b] Number of cycles.

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REFERENCES

1. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005;352: 1223–36.
2. Franks S. Polycystic ovary syndrome. N Engl J Med 1995;333:853–61.
3. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 1999;22:141–6.
4. Baillargeon JP, Nestler JE. Polycystic ovary syndrome: a syndrome of ovarian hypersensitivity to insulin? J Clin Endocrinol Metab 2006;91:22–4.
5. Burghen GA, Givens JR, Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. J Clin Endocrinol Metab 1980;50:113–6.
6. Larner J. Multiple pathways in insulin signalling-fitting the covalent and allosteric puzzle pieces together. Endocr J 1994;2:167–71.
7. Baillargeon JP, Kandarakis ED, Ostlund RE, Apridonidze T, Iuorno MJ, Nestler JE. Altered D-chiro-inositol urinary clearance in women with polycystic ovary syndrome. Diabetes Care 2006;29:300–5.
8. Iuorno MJ, Jacubowicz DJ, Baillargeon JP, Dillon P, Gunn RD, Allan G, Nestler JE. Effect of D-*chiro*-inositol in lean women with the polycystic ovary syndrome. Endocr Pract 2002;8:417–23.
9. Hasegawa I, Murakawa H, Suzuky M, Yamamoto Y, Kurabayashi T, Tanaka T. Effect of troglitazone on endocrine and ovulatory performance in women with insulin-related polycystic ovary syndrome. Fertil Steril 1999;71:323–7.
10. 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;340:1314–20.
11. Gerli S, Mignosa M, Di Renzo GC. Effects of inositol on ovarian function and metabolic factors in women with PCOS: a randomized double blind placebo-controlled trial. Eur Rev Med Pharmacol Sci 2003;7:151–9.
12. Ng EH, Wat NM, Ho PC. Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomized, double-blinded placebo-controlled trial. Hum Reprod 2001;16:1625–31.
13. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, et al. Clomiphene, metformin, or both for infetility in polycystic ovary syndrome. N Engl J Med 2007;356:551–66.
14. Pesant MH, Baillargeon JP. Ovulation induction in polycystic ovary syndrome—how do metformin and clomiphene citrate compare? Nat Clin Pract Endocr Metab 2007;7:512–3.
15. Lord JM, Flight IHK, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 2003;327:951–7.
16. Yoshida K, Yamaguchi M, Morinaga T, Ikeuchi M, Kinehara M, Ashida H. Genetic modification of *Bacillus subtilis* for production of D-*chiro*-inositol, an investigation drug candidate for treatment of type 2 diabetes and polycystic ovary syndrome. Appl Environ Microbiol 2006;72:1310–5.
17. Chiu TT, Rogers MS, Law ELK, 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;17:1591–6.
18. Chiu TTY, Rogers MS, Briton-Jones C, Haines C. Effect of *myo*-inositol on the in-vitro maturation and subsequent development of mouse oocytes. Hum Reprod 2003;18:408–16.
19. Borini A, Sciajno R, Bianchi V, Sereni E, Flamigni C, Coticchio G. Clinical outcome of oocyte cryopreservation after slow cooling with a protocol utilizing a high sucrose concentration. Hum Reprod 2006; 21:512–7.
20. Van de Velde H, Nagy ZP, Joris H, De Vos A, Van Steirteghem AC. Effects of different hyaluronidase concentrations and mechanical procedures for cumulus cell removal on the outcome of intracytoplasmic sperm injection. Hum Reprod 1997;12:2246–50.
21. De Santis L, Cino I, Rabellotti E, Papaleo E, Calzi F, Fusi FM, et al. Oocyte cryopreservation: clinical outcome of slow-cooling protocols differing in sucrose concentration. Reprod Biomed Online 2007;14:57–63.
22. Tummon I, Gavrilova-Jordan L, Allemand MC, Session D. Polycystic ovaries and ovarian hyperstimulation syndrome: a systematic review. Acta Obstet Gynecol Scand 2005;84:611–6.
23. Battaglia C, Mancini F, Persico N, Zaccaria V, De Aloysio D. Ultrasound evaluation of PCO, PCOS and OHSS [Review]. Reprod Biomed Online 2004;9:614–9.
24. Goud PT, Goud AP, Oostveldt PV, Dhont M. Presence and dynamic redistribution of type I inositol, 1,4,5-triphosphate receptor in human oocytes and embryos during in-vitro maturation, fertilization and early cleavage division. Mol Hum Reprod 1999;5:441–51.
25. Papaleo E, Unfer V, Baillargeon JP, De Santis L, Fusi FM, Brigante C, et al. *Myo*-inositol and polycystic ovary syndrome: a novel method for ovulation induction. Gynecol Endocrinol 2007;10:700–3.
26. Veeck LL. Oocyte assessment and biological performance. Ann NY Acad Sci 1988;541:259–74.