CEOG Clinical and Experimental Obstetrics & Gynecology

Original Research

Myoinositol supplementation on intracytoplasmic sperm injection outcome in Japanese infertile polycystic ovarian syndrome women with non-obese less-androgenic phenotype: a prospective controlled observational study

K. Kitaya[1],Y. Takaya[1],R. Nishiyama[1], K. Yamaguchi[1],H. Matsubayashi[1],T. Takeuchi[1],S. Mizuta[1],T. Ishikawa[1]

[1]*Reproduction Clinic Osaka, Osaka, Japan.*

Summary

Purpose of Investigation: The objective of this prospective controlled observational study was to evaluate the effect of myoinositol supplementation on intracytoplasmic sperm injection (ICSI) outcome in Japanese infertile polycystic ovarian syndrome (PCOS) women with non-obese less-androgenic phenotype, which is common in East Asia. *Materials and Methods:* The ICSI outcome in the first treatment cycle under a flexible gonadotropin-releasing hormone antagonist protocol was compared between 25 PCOS women taking 4 g/day myoinositol + 400 μg/day folic acid (Myo/FA group) and 25 PCOS women taking 400 μg/day FA alone (FA Group). *Results:* The total dosage of human menopausal gonadtropin was significantly lower in the Myo/FA group than in the FA group ($p = 0.034$). The number ($p = 0.000029$) and rate (relative risk 1.47, 95% confidence interval 1.43-1.50, $p < 0.0001$) of metaphase II oocytes and the number of fertilized oocytes ($p = 0.0074$) was significantly higher in the Myo/FA group than in the FA group. *Conclusion:* Myoinositol supplementation is a safe and effective treatment modality to increase the mature and fertilized oocytes, along with a reduction in the gonadotropin dose in ICSI cycles in Japanese infertile PCOS women with non-obese less-androgenic phenotype.

Key words: Infertility; Metaphase II oocyte; Fertilization; Myoinositol supplementation; Polycystic ovarian syndrome.

Introduction

Inositols are a group of sugar alcohols, which are subdivided into nine stereoisomers according to the positioning of the hydroxyl group. The most abundant inositol stereoisomer in mammalian cells is myoinositol [(1R,2R,3S,4S,5R,6S)-cyclohexane-1,2,3,4,5,6-hexol], which forms phosphatidylinositol with glycerol and fatty acids in the plasma membrane. Myoinositol is capable of modulating fat and lipid metabolic pathways involved in the signaling-transduction cascade of insulin and serves as a secondary transmitter of intracellular activity of follicle-stimulating hormone. Myoinositol comprises up to 99% of the total inositol amount in the human ovaries and is thought to play a role in oocyte development, cortical granular exocytosis, polyspermy prevention, and meiosis resumption [1].

Polycystic ovarian syndrome (PCOS) is a pathologic condition characterized by chronic anovulation/oligo-ovulation, hyperandrogenism, and ultrasonic polycystic ovarian features. PCOS affects up to 10% of women of reproductive age and is one of the common causes of female infertility [2]. Studies found that the hyperactivity of epimerase in ovarian theca cells in women with PCOS is associated with a consistent myoinositol deficiency and is responsible for anovulation/oligo-ovulation and low oocyte quality in this pathologic condition [3, 4]. This theory is supported by the positive correlation between the follicular fluid myoinositol concentration and oocyte quality [5]. Indeed, myoinositol supplementation is emerging as a promising tool to improve the ovulation disorders, oocyte quality, and pregnancy outcome in infertile PCOS women in Western countries [6].

PCOS has been known to display racial, ethnic, and geographical variations in its prevalence and phenotype. Studies demonstrated that Asian women have a lower prevalence of PCOS compared to other populations. For example, according to Rotterdam 2003 criteria, PCOS was diagnosed in 16% of Danish women and in 21% of indigenous Australian women, whereas it was identified in 5.6% of Chinese women and 6% of Sri Lankan native women [7-10]. In addition, PCOS women in East Asian countries are characterized by low body mass index and mild androgenic phenotype, and the highest prevalence of metabolic syn-

Revised manuscript accepted for publication January 9, 2018

Published: 10 August 2019

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLVI, n. 4, 2019
doi: 10.12891/ceog4567.2019

7847050 Canada Inc.
www.irog.net

Table 1. — *Demographics and baseline characteristics of infertile PCOS patients.*

	Myo/FA group (n = 25)	FA group (n = 25)	*p* value (RR, 95% CI)
Age (years, mean ± SD)	32.1 ± 3.7	31.6 ± 4.1	0.71
BMI (kg/m^2, mean ± SD)	22.0 ± 1.6	22.4 ± 2.8	0.56
Cigarette smoking habit (%)	4	0	> 0.90
Alcohol drinking habit (%)	24	32	> 0.90
Virilization (hirsutism, voice deepening, acne, clitoromegaly)(%)	0	0	> 0.90
Gravidity (median and range, in parenthesis)	0 (0-1)	0 (0-1)	> 0.90
Parity (median and range, in parenthesis)	0 (0-1)	0 (0-1)	> 0.90
Basal follicular stimulating hormone value (IU/l, mean ± SD)	5.6 ± 1.8	5.6 ± 1.7	> 0.90
Basal luteinizing hormone value (IU/l, mean ± SD)	9.5 ± 2.9	8.7 ± 1.8	0.44
Basal prolactin value (ng/ml, mean ± SD)	17.1 ± 8.6	16.7 ± 6.6	0.88
Antral follicle count (mean ± SD)	15.5 ± 4.0	14.3 ± 3.8	0.30
Anti-Müllerian hormone value (ng/ml)	7.8 ± 3.6	7.1 ± 2.1	0.46

SD: standard deviation; RR: relative risk; CI: confident interval.

Table 2. — *ICSI outcome of infertile PCOS patients.*

	Myo/FA group (n = 25)	FA group (n = 25)	*p* value (RR, 95% CI)
Duration of controlled ovarian stimulation (days, median and range)	8 (8-10)	8 (8-11)	> 0.90
Total hMG dosage (IU)	1748 ± 306	1937 ± 298	0.034
Peak estradiol concentration (pg/ml, mean ± SD)	3088 ± 1067	3154 ± 1320	0.85
Onset of moderate/severe ovarian hyperstimulation syndrome	0	0	> 0.90
Total motile sperm counts	6.1 ± 2.4 × 10^6	6.5 ± 3.1 × 10^6	0.55
Number of oocytes retrieved (mean ± SD)	19.6 ± 7.0	17.5 ± 6.4	0.26
Number of metaphase II oocytes retrieved (mean ± SD)	14.5 ± 4.3	8.8 ± 4.3	0.000029
Metaphase II oocyte rate (%)	73.7% (362/491)	50.2% (219/436)	< 0.0001 (1.47, 1.43-1.50)
Number of fertilized oocytes (mean ± SD)	8.2 ± 3.2	5.1 ± 2.8	0.000739
Fertilization rate (%)	56.9% (206/362)	58.4% (128/219)	0.49 (0.97, 0.90-1.05)
Number of morphologically good day-5 blastocysts (mean ± SD)	3.6 ± 2.4	2.4 ± 2.2	0.083
Blastocyst rate (%)	43.2% (89/206)	46.9% (60/128)	0.096 (0.92, 0.83-1.01)

SD: standard deviation; RR: relative risk; CI: confident interval.

drome, which are distinct from those in other regions [11].

Few studies so far reported the supplementation and effect of myoinositol on infertile PCOS women in East Asia. The aim of this prospective study was to investigate the safety and effectiveness of myoinositol supplementation on intracytoplasmic sperm injection (ICSI) outcome in Japanese infertile PCOS women with non-obese less-androgenic phenotype.

Materials and Methods

This was a prospective controlled observational study approved by the Ethical Committee of the Institutional Review Board (Approval Number 2016-1) on February 28th, 2017 and registered on UMIN-CTR Japan (000026393) on March 4th, 2017. The inclusion criteria were as follows: PCOS women 24-40 years of age, body mass index less than 25, and undergoing the first ICSI treatment cycle due to male factor infertility. The exclusion criteria were as follows: the presence of hydrosalpinx, advanced endometriosis, uterine cavity distortion, and any endocrine and metabolic disorders including virilization (hirsutism, voice deepening, acne, and clitoromegaly), hyperprolactinemia, diabetes and thyroid dysfunction. Under an informed consent, a total of 50 women were enrolled in the study.

Flexible gonadotropin-releasing hormone antagonist protocol was adopted for controlled ovarian stimulation. Intramuscular injection of 150-300 IU/day human menopausal gonadotropin (hMG) was initiated on day 3 of the cycle. On the same day, 25 women initiated 4 g/day myoinositol plus 400 μg/day folic acid supplementation (Myo/FA group), while 25 women took 400 μg/day folic acid alone (FA group). Subcutaneous cetrorelix acetate injection was begun when a leading follicle reached a maximal diameter of 14 mm. On the day that two or more leading follicles reached a maximal diameter of 18 mm, 5,000 IU human chorionic gonadotropin was administrated intramuscularly. Transvaginal ultrasound-guided oocyte pickup was performed 35-36 hours following human chorionic gonadotropin trigger. After preincubation for 2-3 hours, the oocytes were subjected to cumulus cell removal, maturation assessment, and ICSI. On the next day, fertilization was confirmed by the presence of two pronuclei [12]. The developing blastocysts were morphologically evaluated according to Gardner's scoring system [13].

The data sets were evaluated for normal distribution using χ^2 goodness-of-fit test and then compared using Student *t*-test, nonparametric Mann-Whitney U test, or Fisher's exact test. The differences were considered as statistically significant when the two-sided *p* value was less than 0.05.

Results

There were no significant differences in the demographics of the infertile couples including age, body mass index, parity, and basal hormonal values (follicular stimulating hormone and luteinizing hormone) between the Myo/FA group and FA group (Table 1). Hyperprolactinemia was di-

agnosed in two patients in the Myo/FA group and in one patient in the FA group, and treated with oral cabergoline administration (0.25 mg tablet, once per week) prior to the initiation of the controlled ovarian stimulation/oocyte pickup cycles. Ovarian reserve markers (antral follicle count on cycle day 3 and serum anti-Müllerian hormone value) were comparable between the groups. Any adverse events including moderate-to-severe ovarian hyperstimulation syndrome, virilization, and drop-out were not seen in both groups.

While the duration of controlled ovarian stimulation and peak estradiol concentration were similar between the two groups (Table 2), the total dosage of hMG was lower in the Myo/FA group than in the FA group (1748 ± 306 IU *vs.* 1937 ± 298 IU, $p = 0.034$). The number of oocytes retrieved was similar between the two groups, whereas the number (14.5 ± 4.3 *vs.* 8.8 ± 4.3, $p = 0.000029$) and rate (73.7% *vs.* 50.2%, relative risk 1.47, 95% confidence interval 1.43-1.50, $p < 0.0001$) of metaphase II oocytes following cumulus removal, were higher in the Myo/FA group than in the FA group. All metaphase II oocytes were subjected to ICSI in both groups.

The number of fertilized oocytes (8.2 ± 3.2 *vs.* 5.1 ± 2.8, $p = 0.0074$) was also higher in the Myo/FA group than in the FA group, although the rate of fertilization was comparable between the groups (56.9% *vs.* 58.4%, relative risk 0.97, 95% confidence interval 0.90-1.05, $p = 0.49$). The number (3.6 ± 2.4 *vs.* 2.4 ± 2.2, $p = 0.083$) and rate (43.2% *vs.* 46.9%, relative risk 0.92, 95% confidence interval 0.83-1.01, $p < 0.096$) of morphologically good blastocysts (Score 3BB or above on day-5 following ICSI) were similar between the groups.

Discussion

Myoinositol supplementation draws much attention in the treatment and management of an-/oligo-ovulatory, and infertile women with PCOS. Myoinositol supplementation was found to improve metabolic and endocrine disorders in PCOS patients by lowering serum luteinizing hormone/follicle-stimulating hormone ratio, circulating androgen/prolactin concentration, correct menstrual cycle irregularity, insulin resistance, and serum sex hormone-binding protein levels [6]. Furthermore, studies focus on a potential role of myoinositol in oocyte differentiation and fecundation in controlled ovarian stimulation cycles [14].

Retrieval of metaphase II oocytes is a prerequisite for successful ICSI treatment. Several approaches such as dehydroepiandrosterone supplementation and double trigger (using gonadotropin-releasing hormone and human chorionic gonadotropin) have been utilized to increase the number and rate of metaphase II oocytes [15, 16]. These methods, however, are potential risk factors for ovarian hyperstimulation syndrome and virilization in infertile patients with PCOS.

In this study, the authors demonstrates that myoinositol supplementation increased the number and rate of the metaphase II oocytes obtained in ICSI cycles of Japanese infertile PCOS women with non-obese less-androgenic phenotype. The number of fertilized oocytes was also higher following myoinositol supplementation. The findings suggest a supportive role of myoinositol in follicular expansion, and possibly in oocyte development. In addition, myoinositol supplementation reduced the total dosage of hMG in PCOS patients compared with control group. Myoinositol supplementation may be an advantageous option to lower the cost and burden of the injections in these women in controlled ovarian stimulation cycles. Meanwhile, the number and rate of morphologically good day-5 blastocysts were similar between the groups. This may result from the study design that enrolled the couples undergoing ICSI only. More detailed studies are required to clarify the effect of myoinositol on embryo culture and development.

Conclusion

Accumulating evidence demonstrated that myoinositol supplementation is a beneficial treatment modality to improve ovulation disorders in infertile Western PCOS women. The authors here first report that myoinositol increased the mature and fertilized oocytes in infertile women with non-obese less-androgenic PCOS, unique to East Asia. Further studies are warranted to provide evidence of myoinositol supplementation effect on their pregnancy outcome.

References

[1] Milewska E.M., Czyzyk A., Meczekalski B., Genazzani A.D.: "Inositol and human reproduction. From cellular metabolism to clinical use". *Gynecol. Endocrinol.,* 2016, *32*, 690.

[2] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group.: "Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome". *Fertil. Steril.,* 2004, *81*, 19.

[3] Heimark D., McAllister J., Larner J.: "Decreased myo-inositol to chiro-inositol (M/C) ratios and increased M/C epimerase activity in PCOS theca cells demonstrate increased insulin sensitivity compared to controls". *Endocrine. J.,* 2014, *6*, 1111.

[4] Unfer V., Carlomagno G., Papaleo E., Vailati S., Candiani M., Bail largeon J.P.: "Hyperinsulinemia alters myoinositol to d-chiroinositol ratio in the follicular fluid of patients with PCOS". *Reprod. Sci.,* 2014, *21*, 854.

[5] Chiu T.T., Rogers M.S., Law E.L., Briton-Jones C.M., Cheung L.P., Haines C.J.: "Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality". *Hum. Reprod.,* 2002, *17*, 1591.

[6] Pundir J., Psaroudakis D., Savnur P., Bhide P., Sabatini L. Teede H., *et al.*: "Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials". *BJOG,* 2018, *125*, 299.

[7] Li R., Zhang Q., Yang D., Li S., Lu S., Wu X.: "Prevalence of polycystic ovary syndrome in women in China: a large community-based study". *Hum. Reprod.,* 2013, *28*, 2562.

[8] Kumarapeli V., Seneviratne Rde A., Wijeyaratne C.N., Yapa R.M., Dodampahala S.H.:"A simple screening approach for assessing community prevalence and phenotype of polycystic ovary syndrome in a semi-urban population in Sri Lanka." *Am. J. Epidemiol.,* 2008, *168*, 321.

[9] Lauritsen M.P., Bentzen J.G., Pinborg A., Loft A., Forman J.L., Thuesen L.L., *et al.*: "The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Mullerian hormone. *Hum. Reprod.,* 2014, *29*, 791.

[10] Boyle J.A., Cunningham J., O'Dea K., Dunbar T., Norman R.J.: "Prevalence of polycystic ovary syndrome in a sample of indigenous women in Darwin, Australia". *Med. J. Aust.,* 2012, *196*, 62.

[11] Zhao Y., Qiao J.: "Ethnic differences in the phenotypic expression of polycystic ovary syndrome". *Steroids,* 2013, *78*, 755.

[12] Kitaya K., Matsubayashi H., Takaya Y., Nishiyama R., Yamaguchi K., Takeuchi T. *et al.*: "Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure". *Am. J. Reprod. Immunol.,* 2017, *78*, e12719.

[13] Gardner D.K., Lane M., Stevens J., Schlenker T., Schoolcraft W.B.: "Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer". *Fertil. Steril.,* 2000, *73*, 1155.

[14] Nestler J. E., Jakubowicz D. J., Reamer P., Gunn R. D., Allan G.: "Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome." *N. Engl. J. Med*., 1999, *340*, 1314.

[15] Haas J., Ophir L., Barzilay E., Machtinger R., Yung Y., Orvieto R., Hourvitz A.: " Standard human chorionic gonadotropin versus double trigger for final oocyte maturation results in different granulosa cells gene expressions: a pilot study." *Fertil. Steril.,* 2016, *106*, 653.

[16] Nagels H.E., Rishworth J.R., Siristatidis C.S., Kroon B.: "Androgens (dehydroepiandrosterone or testosterone) for women undergoing assisted reproduction." *Cochrane. Database. Syst. Rev*., 2015, *11*, CD009749.

[17] Pacchiarotti A., Carlomagno G., Antonini G., Pacchiarotti A.: "Effect of myo-inositol and melatonin versus myo-inositol, in a randomized controlled trial, for improving *in vitro* fertilization of patients with polycystic ovarian syndrome." *Gynecol. Endocrinol*., 2016, *32*, 69.

Corresponding Author:
K. KITAYA, M.D.
Reproduction Clinic Osaka
Grand Front Osaka 15F
10-15, Oofuka-cho, kita-ku
Osaka City 530-0011 (Japan)
e-mail: kitaya@koto.kpu-m.ac.jp