Inositol and In Vitro Fertilization with Embryo Transfer

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Recently, studies on inositol supplementation during in vitro fertilization program (IVF) have gained particular importance due to the effect of this molecule on reducing insulin resistance improving ovarian function, oocyte quality, and embryo and pregnancy rates and reducing gonadotropin amount during stimulation. Inositol and its isoforms, especially myoinositol (MYO), are often used as prestimulation therapy in infertile patients undergoing IVF cycle. Inositol supplementation started three months before ovarian stimulation, resulting in significant improvements in hormonal responses, reducing the amount of FSH necessary for optimal follicle development and serum levels of 17beta-estradiol measured the day of hCG injection. As shown by growing number of trials, MYO supplementation improves oocyte quality by reducing the number of degenerated and immature oocytes, in this way increasing the quality of embryos produced. Inositol can also improve the quality of sperm parameters in those patients affected by oligoasthenoteratozoospermia.

1. Introduction

Despite 30 years of history, assisted reproduction technologies (ART) still present many challenges in order to identify factors and predictors of success.

It is well known that oocyte quality is the main factor determining chance of pregnancy and that poor quality is an obstacle for successful in vitro fertilization results. It has become increasingly clear that the follicular microenvironment of a human oocyte is a crucial factor for its developmental competence [1].

Through the years, many studies have been proposed to find strategies, drugs, or compounds such as antioxidant drugs and supplementation with vitamins or hormones able to improve oocyte quality and embryo quality [2].

Recently, studies on inositol supplementation during in vitro fertilization program (IVF) have gained particular importance due to the effect of this molecule on reducing insulin resistance improving ovarian function, oocyte quality, and embryo and pregnancy rates and reducing gonadotropin amount during stimulation [3]. Inositol and its isoform, especially myoinositol, find their application as prestimulation therapy in polycystic ovary syndrome (PCOS) patients undergoing IVF cycle and, recently, also in all kinds of infertile patients such as poor responders [4].

Studies demonstrate that the use of inositol in male patients affected by oligoasthenoteratozoospermia can improve sperm cell parameters and consequently the impact of fertilization rate and embryo quality leading to high percentage of pregnancy [5].

2. Inositol

Inositol (cyclohexanehexol) is a cycle polyol commonly referred to as a B vitamin, although not a true vitamin. It is widely distributed in human tissue and cells, and it is a precursor for phosphorylated compounds known as phosphoinositides which are involved in signal transduction through membrane receptor stimulation and other secondary messengers including diacylglycerol (DAG) and inositol triphosphate (IP3) that can be located at the inner or outer side of membrane and are involved in insulin transduction signaling. DAG activates protein kinase C (PKC) and IP3 activates intracellular calcium (Ca2+) release, an essential step in oocyte maturation and so of fertilization process. There are nine inositol stereoisomers, and myoinositol is the most represented in cellular content [6]. All stereoisomers act as mediator of insulin action inside the cell [7]: myoinositol (MYO) and D-chiro-inositol (DCI) are inositol-containing...
phosphoglycan (IPG) mediators, generated by hydrolysis of glycosylphosphatidylinositol that inhibits cyclic AMP-dependent protein kinase (the first) and activates pyruvate dehydrogenase (the second) [8].

MYO has been shown to influence different pathways at both ovarian and nonovarian levels. MYO is an important constituent of follicular microenvironment and it plays a determinant role in both nuclear and cytoplasmatic oocyte development [9], being also a precursor of phospholipids, which are responsible for the generation of important intracellular signals oocytes such as release of cortical granules, inhibition of polyspermy, and resumption of meiotic process [10]. Furthermore, MYO seems to significantly modulate steroidogenesis by acting through an insulin-independent pathway that involves cytoskeleton rearrangements [11].

On the contrary, DCI alone is not able to make significant improvements in the ovarian cell functions, as its beneficial effects are mainly confined to the nonovarian tissue in which it may significantly inhibit the negative cellular consequences of hyperinsulinemia. However, both inositol isomers can be effectively used in the management of PCOS patients in a ratio corresponding to their physiological plasma ratio (40:1). This seems to exert a synergistic effect according to a multilayered design [12].

According to DCI ovary paradox theory, an increase of epimerase function in the ovaries causes an increase of DCI level associated with a local MYO deficiency and poor oocyte quality [13] with a negative effect in FSH stimulation and in ovulation [14]. Finally, some studies observed that high dosage of DCI administration may damage oocytes [15].

3. Inositol and In Vitro Fertilization

During the last decades, researches have focused on the role of the two major inositol stereoisomers, MYO and DCI, in particular on the effects of the first on oocyte quality. Among the causes of infertility, PCOS patients undergoing ovarian stimulation are subjected to an increased risk of in vitro fertilization failure due to poor oocyte/embryo quality and/or risk of ovarian hyperstimulation syndrome (OHSS). One of the goals of ovarian stimulation in PCOS is to recover an adequate number of mature oocytes avoiding OHSS. Clinical trials show that MYO supplementation started three months before the onset of ovarian stimulation results in significant improvements in hormonal responses, reducing the international unit (IU) of FSH needed to an optimal follicular fluid in PCOS patients shows a 500-fold reduction of the amount of MYO, associated with an increase of insulin resistance, hyperinsulinemia, and luteinizing hormone levels [30].

Our recent study evaluates the effects of MYO administration on hormonal parameters in PCOS. 50 overweight PCOS patients undergo hormonal evaluations and an oral glucose tolerance test (OGTT) before and after 12 weeks of supplementation with myoinositol. Patients are divided into two groups: one treated with MYO 2 g and folic acid 200 µg daily (Group A) and the other one receiving only folic acid 400 mg (Group B-controls). Ultrasound examinations and Ferriman-Gallwey score are also performed. We note that after 12 weeks of MYO administration plasma
Few data are available, at the moment, about the effects of MYO supplementation on in vitro fertilization outcome in sterile patients not affected by polycystic ovary syndrome. Recently, Lisi et al. have examined the effects of inositol administration on oocyte and embryo quality in infertile women undergoing IVF cycle by conventional IVF or intracytoplasmic sperm injection (ICSI). One hundred non-PCOS patients aged under 40 years and with basal FSH < 10 mIU/ml undergoing ovarian stimulation are randomly divided into two groups: Group A treated with 400 μg of folic acid for the 3 months before and during rFSH administration and Group B treated with a daily dose of 4000 mg of myoinositol into two administrations/day in addition to 400 μg of folic acid for the 3 months before and during rFSH administration. Group B shows a reduction in the number of mature oocytes retrieved and in the amount of gonadotropins used, whereas implantation rate and clinical pregnancy rate are improved [4].

The effect of MYO supplementation on ovarian function has also been evaluated in poor responders patients [33] undergoing ICSI. The study involves 76 poor responders divided into two groups: 38 patients who have been assuming MYO (4 g) plus folic acid (400 μg) for the previous 3 months before the start (Group A) and 38 patients assuming only folic acid (FA) (400 μg) for the same period (Group B). Ovarian stimulation is carried out with a GnRH antagonist protocol in both groups. They do not observe any significant difference between the two groups regarding estradiol level, but total rec-FSH units used are significantly lower (p = 0.004) and metaphase II (MII) oocytes rate is significantly higher (p = 0.01) in Group A. The ovarian sensitivity index is higher, reaching a statistical significance (p < 0.05), in the group of patients pretreated with MYO, showing an improvement in ovarian sensibility to gonadotropin. In conclusion, they suggest that MYO supplementation in poor responder patients results in an increase of the number of oocytes recovered in MII and of the gonadotropin ovarian sensitivity, suggesting a MYO role in improving ovarian response to gonadotropins. Hence, MYO seems to be helpful in poor responders undergoing IVF cycles [34].

### 6. Inositol in Sperm Cell

About the role of MYO in male reproduction, Chauvin and Griswold show that MYO concentration in the seminiferous tubules is higher than in serum [35]; moreover, MYO levels are increased by movement of spermatozoa through epididymis and deferent duct [36]. Patients affected by oligoasthenoteratospermia have spermatozoa totally covered by “amorphous fibrous material,” which reduces sperm mobility. Colone et al. show that MYO administration could help to reduce the presence of this amorphous material [37]. MYO has also a crucial role in the osmoregulation of seminal fluid and as a consequence in sperm progressive motility and velocities [38]. Gulino et al. investigate the effect of MYO administration on semen parameters of male patients undergoing IVF cycles. They collect semen samples of 62 patients divided into three different groups: healthy fertile patients (Group A); patients with oligoasthenospermia (OA) (Group B); and control group (CTR). The first two groups receive administration of 4000 mg/die of MYO and 400 μg/die of folic acid for 2 months. Semen’s volume and spermatozoa’s number and motility are the parameters evaluated before and after treatment and before and after density-gradient separation. Spermatozoa concentrations are higher in both Groups A and B. In conclusion, they showed that MYO supplementation significantly improves semen’s parameters both in patients with OA and in normal fertile men [5].

### 7. Conclusion

Nowadays, many studies demonstrate the positive effects of myoinositol in patients undergoing IVF cycle so it could be a predictive factor in improving ART outcomes. In particular, as revealed by a conference scientific committee, MYO improves both ovarian response to gonadotropins during IVF stimulation and oocyte and embryo quality.
Conflicts of Interest

The authors declare that this research is conducted in the absence of any commercial or financial relationship that can be a potential conflict of interest.

References

[1] P. G. Artini, V. Valentino, P. Monteleone et al., "Vascular endothelial growth factor level changes during human embryo development in culture medium," Gynecological Endocrinology, vol. 24, no. 4, pp. 184–187, 2008.

[2] L. L. van Loendersloot, M. van Wely, J. Limpens, P. M. M. Bossuyt, S. Repping, and F. van der Veen, "Predictive factors in in vitro fertilization (IVF): a systematic review and meta-analysis," Human Reproduction Update, vol. 16, no. 6, pp. 577–589, 2010.

[3] L. Ciotta, M. Stracquadanio, I. Pagano, A. Carbonaro, M. Palumbo, and F. Gulino, "Effects of myo-inositol supplementation on oocyte’s quality in PCOS patients: a double blind trial," European Review for Medical and Pharmacological Sciences, vol. 15, no. 5, pp. 509–514, 2011.

[4] F. Lisi, P. Carfagna, M. M. Oliva et al., "Pretreatment with myo-inositol in non polycystic ovary syndrome patients undergoing multiple follicular stimulation for IVF: a pilot study," Reproductive Biology and Endocrinology, vol. 10, 52 pages, 2012.

[5] F. A. Gulino, E. Leonardi, I. Marilli et al., "Effect of treatment with myo-inositol on semen parameters of patients undergoing an IVF cycle: in vivo study," Gynecological Endocrinology, vol. 32, no. 1, pp. 65–68, 2016.

[6] J. Benjamin, H. Nemetz, M. Fux, I. Bleichman, and G. Agam, "Acute inositol does not attenuate m-CPP-induced anxiety, mydriasis and endocrine effects in panic disorder," Journal of Psychiatric Research, vol. 31, no. 4, pp. 489–495, 1997.

[7] L. C. Huang, M. C. Fonteles, D. B. Houston, C. Zhang, and J. Larner, "Chiroinositol deficiency and insulin resistance. III. Acute glycogenic and hypoglycemic effects of two inositol phosphoglycan insulin mediators in normal and streptozotocin-diabetic rats in vivo," Endocrinology, vol. 132, no. 2, pp. 652–657, 1993.

[8] S. Akiba and T. Sato, "Cellular function of calcium-independent phospholipase A2," Biological & Pharmaceutical Bulletin, vol. 27, no. 8, pp. 1174–1178, 2004.

[9] P. G. Artini, O. M. Di Berardino, F. Papini et al., "Endocrine and clinical effects of myo-inositol administration in polycystic ovary syndrome. A randomized study," Gynecological Endocrinology, vol. 29, no. 4, pp. 375–379, 2013.

[10] S. D. Smith, A. Mikkelson, and S. Lindenberg, "Development of human oocytes matured in vitro for 28 or 36 hours," Fertility and Sterility, vol. 73, no. 3, pp. 541–544, 2000.

[11] M. Bizzarri, A. Cucina, S. Dinicola et al., "Does myo-inositol effect on PCOS follicles involve cytoskeleton regulation?" Medical Hypotheses, vol. 91, pp. 1–5, 2016.

[12] A. Bevilacqua and M. Bizzarri, "Physiological role and clinical utility of inositol in polycystic ovary syndrome," Best Practice & Research Clinical Obstetrics & Gynaecology, vol. 37, pp. 129–139, 2016.

[13] G. Carломagno, V. Unfer, and S. Roseff, "The D-chiro-inositol paradox in the ovary," Fertility and Sterility, vol. 95, no. 8, pp. 2515–2516, 2011.

[14] A. S. Laganà, P. Rossetti, M. Buscema et al., "Metabolism and ovarian function in polycystic ovary syndrome: a therapeutic approach with inositol," International Journal of Endocrinology, vol. 2016, Article ID 6306410, 9 pages, 2016.

[15] R. Isabella and E. Raffone, "Does ovary need D-chiro-inositol?" Journal of Ovarian Research, vol. 5, no. 1, 14 pages, 2012.

[16] E. Papaleo, V. Unfer, J.-P. Baillargeon, F. Fusi, F. Occhi, and L. De Santis, "Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial," Fertility and Sterility, vol. 91, no. 5, pp. 1750–1754, 2009.

[17] V. Unfer, E. Raffone, P. Rizzo, and S. Buffo, "Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous in vitro fertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study," Gynecological Endocrinology, vol. 27, no. 11, pp. 857–861, 2011.

[18] P. Rizzo, E. Raffone, and V. Benedetto, "Effect of the treatment with myo-inositol plus folic acid plus melatonin in comparison with a treatment with myo-inositol plus folic acid on oocyte quality and pregnancy outcome in IVF cycles. A prospective, clinical trial," European Review for Medical and Pharmacological Sciences, vol. 14, no. 6, pp. 555–561, 2010.

[19] T. T. Y. Chiu, M. S. Rogers, E. L. K. Law, C. M. Briton-Jones, L. P. Cheung, and C. J. Haines, "Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality," Human Reproduction (Oxford, England), vol. 17, no. 6, pp. 1591–1596, 2002.

[20] R. Homburg, "Polycystic ovary syndrome—from gynaecological curiosity to multisystem endocrinopathy," Human Reproduction (Oxford, England), vol. 11, no. 1, pp. 29–39, 1996.

[21] Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, "Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS)," Human Reproduction (Oxford, England), vol. 19, no. 1, pp. 41–47, 2004.

[22] A. D. Genazzani, C. Lanzoni, F. Ricchiere, E. Baraldi, E. Casarosa, and V. M. Jasonni, "Metformin administration is more effective when non-obese patients with polycystic ovary syndrome show both hyperandrogenism and hyperinsulinemia," Gynecological Endocrinology, vol. 23, no. 3, pp. 146–152, 2007.

[23] A. Pizzo, A. S. Laganà, and L. Barbaro, "Comparison between effects of myo-inositol and D-chiro-inositol on ovarian function and metabolic factors in women with PCOS," Gynecological Endocrinology, vol. 30, no. 3, pp. 205–208, 2014.

[24] J. E. Nestler, D. J. Jakubowicz, P. Reamer, R. D. Gunn, and G. Allan, "Ovolatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome," The New England Journal of Medicine, vol. 340, no. 17, pp. 1314–1320, 1999.

[25] M. J. Iuorno, D. J. Jakubowicz, J. P. Baillargeon et al., "Effects of D-chiro-inositol in lean women with the polycystic ovary syndrome," Endocrine Practice, vol. 8, no. 6, pp. 417–423, 2002.

[26] S. Gerli, E. Papaleo, A. Ferrari, and G. C. Di Renzo, "Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS," European Review for Medical and Pharmacological Sciences, vol. 11, no. 5, pp. 347–354, 2007.

[27] M. Minozzi, D. Costantini, C. Gualardi, and V. Unfer, "The effect of a combination therapy with myo-inositol and a combined oral contraceptive pill versus a combined oral contraceptive pill alone on metabolic, endocrine, and clinical
parameters in polycystic ovary syndrome,” *Gynecological Endocrinology*, vol. 27, no. 11, pp. 920–924, 2011.

[28] V. Unfer, G. Carломagno, P. Rizzo, E. Raffone, and S. Roseff, “Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial,” *European Review for Medical and Pharmacological Sciences*, vol. 15, no. 4, pp. 452–457, 2011.

[29] S. Colazingari, M. Treglia, R. Najjar, and A. Bevilacqua, “The combined therapy myo-inositol plus D-chiro-inositol, rather than D-chiro-inositol, is able to improve IVF outcomes: results from a randomized controlled trial,” *Archives of Gynecology and Obstetrics*, vol. 288, no. 6, pp. 1405–1411, 2013.

[30] A. Pacchiarotti, G. Carломagno, G. Antonini, and A. Pacchiarotti, “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,” *Gynecological Endocrinology*, vol. 32, no. 1, pp. 69–73, 2016.

[31] M. Aboulghar, “Symposium: update on prediction and management of OHSS. Prevention of OHSS,” *Reproductive Biomedicine Online*, vol. 19, no. 1, pp. 33–42, 2009.

[32] G. A. Turan, F. Eskicioglu, O. N. Sivrikoz et al., “Myo-inositol is a promising treatment for the prevention of ovarian hyper-stimulation syndrome (OHSS): an animal study,” *Archives of Gynecology and Obstetrics*, vol. 292, no. 5, pp. 1163–1171, 2015.

[33] A. P. Ferraretti, A. La Marca, B. C. J. M. Fauser, B. Tarlatzis, G. Nargund, and L. Gianaroli, “ESHRE consensus on the definition of ‘poor response’ to ovarian stimulation for in vitro fertilization: the Bologna criteria,” *Human Reproduction (Oxford, England)*, vol. 26, no. 7, pp. 1616–1624, 2011.

[34] F. Caprio, M. D. D’Eufemia, C. Trotta et al., “Myo-inositol therapy for poor-responders during IVF: a prospective controlled observational trial,” *Journal of Ovarian Research*, vol. 8, 37 pages, 2015.

[35] T. R. Chauvin and M. D. Griswold, “Characterization of the expression and regulation of genes necessary for myo-inositol biosynthesis and transport in the seminiferous epithelium,” *Biology of Reproduction*, vol. 70, no. 3, pp. 744–751, 2004.

[36] B. T. Hinton, R. W. White, and B. P. Setchell, “Concentrations of myo-inositol in the luminal fluid of the mammalian testis and epididymis,” *Journal of Reproduction and Fertility*, vol. 58, no. 2, pp. 395–399, 1980.

[37] M. Colone, G. Marelli, V. Unfer, G. Bozutto, A. Molinari, and A. Stringaro, “Inositol activity in oligoasthenoteratospermia—an in vitro study,” *European Review for Medical and Pharmacological Sciences*, vol. 14, no. 10, pp. 891–896, 2010.

[38] D. Y. Liu, G. N. Clarke, and H. W. G. Baker, “Hyper-osmotic condition enhances protein tyrosine phosphorylation and zona pellucida binding capacity of human sperm,” *Human Reproduction (Oxford, England)*, vol. 21, no. 3, pp. 745–752, 2006.