GYNECOLOGICAL ENDOCRINOLOGY

http://informahealthcare.com/gye
ISSN: 0951-3590 (print), 1473-0766 (electronic)

Gynecol Endocrinol, Early Online: 1–6
 DOI: 10.3109/09513590.2015.1006616

informa healthcare

POSITION STATEMENT

Results from the International Consensus Conference on myo-inositol and D-chiro-inositol in Obstetrics and Gynecology – assisted reproduction technology

Arturo Bevilacqua[1], Gianfranco Carlomagno[2], Sandro Gerli[3], Mario Montanino Oliva[4], Paul Devroey[5], Antonio Lanzone[6], Christophe Soulange[7], Fabio Facchinetti[8], Gian Carlo Di Renzo[3,9], Mariano Bizzarri[10], Moshe Hod[11], Pietro Cavalli[12], Rosario D'Anna[13], Salvatore Benvenga[14], Tony T. Chiu[15], and Zdravko A. Kamenov[16]

[1]Department of Psychology, Sapienza University of Rome, Rome, Italy, [2]Lo.Li. Pharma R&D Department, Rome, Italy, [3]Department of Obstetrics and Gynecology, University of Perugia, Perugia, Italy, [4]Center for Reproductive Medicine Research, Clinica Villa Mafalda, Rome, Italy, [5]Center for Reproductive Medicine, Dutch-Speaking Free University Brussels, Brussels, Belgium, [6]Institute of Obstetrics and Gynecology, Catholic University of the Sacred Heart, Rome, Italy, [7]INSA de Lyon, CarMeN, INSERM U1060, Université de Lyon, Villeurbanne, France, [8]Mother–Infant Department, University of Modena and Reggio Emilia, Modena, Italy, [9]PREIS School, Milan, Italy, [10]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy, [11]Helen Schneider Hospital for Women, Rabin Medical Center, Petah Tikva, Israel, [12]Servizio di Genetica, Istituti Ospedalieri di Cremona, Cremona, Italy, [13]Department of Obstetrics and Gynecological Sciences, University of Messina, Messina, Italy, [14]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy, [15]Hong Kong Reproductive Medicine Centre, Hong Kong, People's Republic of China, and [16]Clinic of Endocrinology, Alexandrovska University Hospital, Medical University, Sofia, Bulgaria

Abstract

A substantial body of research on mammalian gametogenesis and human reproduction has recently investigated the effect of myo-inositol (MyoIns) on oocyte and sperm cell quality, due to its possible application to medically assisted reproduction. With a growing number of both clinical and basic research papers, the meaning of several observations now needs to be interpreted under a solid and rigorous physiological framework. The 2013 Florence International Consensus Conference on Myo- and D-chiro-inositol in obstetrics and gynecology has answered a number of research questions concerning the use of the two stereoisomers in assisted reproductive technologies. Available clinical trials and studies on the physiological and pharmacological effects of these molecules have been surveyed. Specifically, the physiological involvement of MyoIns in oocyte maturation and sperm cell functions has been discussed, providing an answer to the following questions: (1) Are inositols physiologically involved in oocyte maturation? (2) Are inositols involved in the physiology of spermatozoa function? (3) Is treatment with inositols helpful within assisted reproduction technology cycles? (4) Are there any differences in clinical efficacy between MyoIns and D-chiro-inositol? The conclusions of this Conference, drawn depending on expert panel opinions and shared with all the participants, are summarized in this review paper.

Keywords

Infertility, polycystic ovary syndrome, pregnancy, ovary, ovulation induction

History

Received 11 December 2014
Accepted 8 January 2015
Published online 2 June 2015

Introduction

Medically assisted reproduction techniques now have a history of 30 years and all the studies carried out so far agree on the importance of identifying the quality of the oocyte as the main predictor of success [1,2]. Although the most important factor influencing oocyte quality is the age of the woman, today many couples tend to delay the arrival of their first child, and this phenomenon is the leading cause of couple infertility with significant medical, psychological and social consequences. To overcome this problem, many studies have tried to identify compounds able to improve the quality of oocytes [3] and a growing number of observations have focused on myo-inositol (MyoIns), because (a) its concentration in the follicular fluid directly correlates with the quality of oocytes and embryos [4] and (b) *in vitro* fertilization (IVF) cycles, treatment of women with MyoIns before the hormonal stimulation reduces the amount of FSH and days required for proper stimulation, parameters directly related to the possibility of pregnancy [5], improves quality of oocytes [6–9] and embryos [6,10] and, probably, the implantation rate [11]. Due to these effects, MyoIns has found application in a number of clinical trials carried out on patients with polycystic ovary syndrome (PCOS) [6–12], which reduces the quality of gametes and represents the leading cause of infertility in young women [13,14]. Results obtained suggest that MyoIns may be routinely used in the treatment of women approaching IVF

Address for correspondence: Arturo Bevilacqua, Department of Psychology, Sapienza University of Rome, via dei Marsi 78, 00185 Rome, Italy. Tel: +39 0649917624. E-mail: arturo.bevilacqua@uniroma1.it

techniques in light of their low quality oocytes and independent from PCOS.

Besides its effects on oocyte quality, observations obtained on male subjects suffering from oligoasthenoteratozoospermia (OAT), a serious medical condition that impairs sperm cell number, morphology and function, have shown that MyoIns increases sperm cell parameters [15].This suggests that its use in the treatment of semen samples in IVF cycles would positively impact on fertilization rate and embryo quality, therefore leading to higher chances of pregnancy.

In parallel to the increasing clinical data on humans, experiments conducted on laboratory and farming species have long suggested a role for MyoIns in oogenesis and preimplantation mammalian development. Indeed, supplementation of culture media with MyoIns has positive effects on meiotic maturation of mouse oocytes [16] and the completion of preimplantation development of bovine, rabbit and mouse embryos [17–19], also allowing the development to term of healthy animals [16,17]. These observations have prompted the hypothesis that the inclusion of this molecule in culture media for human embryos can produce an increase in the number of high quality embryos obtained in IVF cycles.

MyoIns

General features of MyoIns are well known, along with those of its nine stereoisomers, all belonging to the family of inositols with formula $C_6H_{12}O_6$. Inositols are broadly distributed in mammalian tissues and cells where they perform important biologic functions. The cellular content of inositol is represented almost entirely by MyoIns (>99%) and for the remaining part by a second stereoisomer, D-chiro-inositol (DCIns). Although having different metabolic functions, both of these molecules are mediators of insulin action inside the cell [20]. MyoIns is converted into DCIns by the enzyme epimerase, the tissue specific expression of which influences concentration ratios observed between the two molecules in different cells of an organism, such as hepatic or adipose cells, or muscle fibers [21].

Inside a cell, MyoIns is not only present as free form, but also as a component of membrane phosphoinositides, including phosphatidyl-inositol phosphate and phosphatidyl-inositol bisphosphate (PIP_2), molecules with important physiological roles [22]. Hydrolysis of PIP_2 by phospholipase C (PLC) produces inositol-trisphosphate (Ins-1,4,5P_3, InsP_3), which regulates activities of hormones that include FSH, TSH and insulin as a second messenger [22]. By interaction with membrane receptors of mitochondria and the endoplasmic reticulum, InsP_3 induces calcium influx into the cytosol, which activates protein kinase C and mediates cellular responses. Other membrane lipids containing inositol (glycosyl-phosphatidylinositol) serve as anchor for many membrane proteins.

MyoIns is involved in processes including glucose metabolism and the regulation of cell proliferation [23,24], relevant during development in all its phases [25–28]: pre- as well as postimplantation embryogenesis and, in the adult, oogenesis and spermatogenesis.

Conference purpose and methods

As proven by systematic reviews or Cochrane reviews mixing trials performed using MyoIns or DCIns, the knowledge of the differences between the two molecules in the scientific community is not well established yet. In order to place a milestone and to open some reflection points on this issue, the PREIS School (Permanent International and European School in Perinatal Neonatal and Reproductive Medicine) has organized the ''2013 Florence International Consensus Conference on Myo and D-chiro-inositol in obstetrics and gynecology''. To this end, the PREIS Chairman, G. D. R., identified opinion leaders in the fields of cell biology (G. C., M. B., C. S., P. C.), mammalian embryology (A. B., T. T. C.), human endocrinology (A. L., S. B.), metabolism (R. D. A., Z. A. K.), obstetrics and gynecology (S. G., M. M. O., P. D., F. F., M. H.), who have eventually become involved in research studies of inositols and its clinical and cellular effects. The Consensus Conference scientific Committee, divided into two separate panels, reviewed updated information on the roles of MyoIns and DCIns in metabolism, obstetrics and gynecology on one side and in assisted reproduction technology on the other side.

In particular, the second issue was discussed by answering the following questions: Are inositols physiologically involved in oocyte maturation? Are inositols involved in the physiology of spermatozoa function? Is treatment with inositol(s) helpful within IVF cycles? Are there any differences in clinical efficacy between MyoIns and DCIns?

In order to answer these questions, the panel examined seminal papers on the role of MyoIns in mammalian/human oocyte and sperm cell physiology, together with clinical research articles in the form of randomized controlled trial and double blind randomized controlled trial, proposed by the Organizing School and selected on the basis of each participant's knowledge/experience. A preliminary statement containing the panel's recommendations was drawn during the Conference, which represents, unmodified, the basis of the present, conclusive paper, redacted by A. B., S. G. and F. F. according to the guidelines of the Italian Ministry of Health and National Institute of Health.

Are inositols physiologically involved in oocyte maturation?

Role of MyoIns in oogenesis and early embryogenesis

The concentration of MyoIns in mammalian female reproductive tracts [29] is substantially higher than that of blood serum, and this suggests that MyoIns has a positive influence on fertility and a role in reproduction. A positive correlation between MyoIns content in the follicular fluid and oocyte quality and pregnancy outcome has been demonstrated in humans [4,30].

MyoIns has indeed different functions at the ovarian level, being essential to ensure proper oocyte maturation. The action of this molecule is related to the role played by InsP_3 on the modulation of intracellular calcium ion concentration in response to the action of the hormones LH and FSH [31,32]. In oocytes, this mechanism involves specific receptors (InsP_3-R1) [33] and appears to play a key role in the maturation process [34].

Culture medium supplementation with MyoIns increases meiotic progression of mouse oocytes with the production of fertile eggs, while the depletion of intracellular stores of MyoIns desensitizes inositol-dependent transductions pathways, reducing levels of InsP_3 and the proper release of calcium and reduces oocyte maturation [16]. When oocytes matured in the presence of MyoIns are fertilized *in vitro* and transferred to foster mothers, the implantation rate and postimplantation viability of the resulting embryos is also increased [16].

MyoIns is actively imported into mammalian cells, including oocytes and preimplantation embryos. During early development of the mouse, activity of at least two different membrane protein transporters [35,36] allows a progressively increasing uptake of MyoIns between the one-cell stage and the blastocyst stage [36,37]. MyoIns is then rapidly incorporated into phosphoinositides [37]. In the zygote, concentration oscillations of calcium ions, induced by PLC-dependent InsP_3 production, have key roles, from egg activation at fertilization [38] to blastomere divisions [39]. The inclusion of MyoIns in human embryo culture media has

recently been shown to increases the ability of *in vitro* produced and cultured mouse embryos to complete preimplantation development [19]. Compared to embryos cultured in standard medium, embryos maintained in the presence of MyoIns displayed a faster cleavage and developmental rate, leading to hypothesize the use of this molecule for human embryo culture in IVF cycles [19].

MyoIns and oogenesis: a lesson from PCOS

PCOS is the most common cause of infertility, ovarian dysfunction and menstrual irregularity, affecting 5–10% of women in reproductive age [40]. The pathogenesis of PCOS has been linked to the development of insulin resistance and hyperinsulinemia, frequently observed in these patients [41,42]. It has been therefore hypothesized that an altered insulin signal transduction in PCOS patients may cause insulin resistance, which in turn induces abnormal ovarian steroidogenesis [43,44]. For this reason, among the treatments routinely used, women with PCOS seem to respond favorably to insulin-sensitizing drugs, including MyoIns and DCIns [45–48].

Some studies have shown that women with PCOS respond to DCIns with an increase in ovarian activity and menstrual frequency [48,49]. However, subsequent studies have shown that MyoIns is more effective than DCIns in the management of patients with PCOS [6,47,50], resulting among others in the induction of regular menstrual cycles [48,50] and in the improvement of oocyte quality [9]. In IVF cycles, treatment of PCOS women with MyoIns before the hormonal stimulation increases the quality of oocytes [6–8] and embryos [6,10] and, as suggested by unconfirmed observations, the rate of implantation [11]. Very recently, analysis of the follicular fluid of PCOS patients has revealed a 500-fold reduction in the amount of MyoIns, accompanied by increased of insulin resistance, hyperinsulinemia and luteinizing hormone levels [51]. It is therefore becoming apparent that MyoIns depletion in the PCOS ovary interferes with dominant follicle recruitment and proper oocyte growth/maturation. These observations confirm seminal observations by Chiu et al. [4], further indicating that proper content of follicular fluid MyoIns is a necessary condition to insure egg quality.

Are inositols involved in the physiology of spermatozoa function?

Similarly to what observed in females, the concentration of MyoIns in mammalian male reproductive tracts is also higher than that of blood serum and increases from the caput to the cauda epididymis [52–54]. In male reproductive organs, MyoIns is mainly produced by FSH-responsive Sertoli cells and is involved in processes that include the regulation of motility, capacitation and acrosome reaction of sperm cells.

Studies performed on pathological sperm samples such as those typical of OAT patients have shown that MyoIns is crucial for at least two different functions, one at the extracellular level and the second one at the intracellular level. Sperm cells of OAT patients are characterized by low motility and higher levels of the enzyme inositol monophosphatase-1 (IMPA-1) [55], responsible for the dephosphorylation of phosphatidylinositol (PI). This sustains the role of signal transduction pathways induced by PI in the regulation and maintenance of male germ cell motility [56]. Besides the reduced motility, sperm cells of OAT patients are characterized by morphological abnormalities in mitochondria and the midpiece as well as the presence of an amorphous-fibrous material covering them entirely and causing their clotting. Electron microscopy imaging has recently demonstrated that treatment of these cells with MyoIns reduces the presence of the amorphous material and semen viscosity [57]. Furthermore, MyoIns improves midpiece volume and restores mitochondrial cristae morphology, suggesting a structural normalization of mitochondria [57].

At the functional level, MyoIns acts directly on mitochondria increasing the membrane potential [58]. Mitochondrial membrane potential is an apoptotic marker clearly related to the functional parameters of the sperm cells, including motility [59] and the capacity of fertilization and embryo quality [60], and is therefore used as an index of fertility. High values of mitochondrial membrane potential indicate integrity of this structure with optimal levels of activity and are associated with high cell viability. Confirming these morphological-functional data, treatment of sperm cells from both OAT patients and normal subjects with MyoIns increases total and progressive motility, improving the recovery of cells usable in IVF cycles after swim-up [15]. These observations strongly suggest a direct impact of MyoIns on fertilization capacity of male germ cells, and favor its use as supplement in their manipulation within medical assisted reproduction procedures.

Is treatment with inositol(s) helpful within ART cycles?

Much consensus on this issue has been gathered regarding women with PCOS. Among other comorbidities, PCOS patients undergoing ovarian stimulation are subjected to an increased risk of failure of the IVF cycle due to poor oocyte/embryo quality and/or increased risk of ovarian hyperstimulation syndrome (OHSS). The clinical trials so far performed show that MyoIns pretreatment beginning three months before the onset of ovarian stimulation results in significant improvements in hormonal responses [6,7,8,12]. In particular, MyoIns reduces the amount of FSH necessary for optimal follicle development as well as serum levels of 17beta-estradiol measured the day of hCG injection. This reduces the chance of OHSS also reducing the number of cancelled cycles. For these reasons, pretreatment of the patients with MyoIns appears really promising.

As for the biological outcomes of these trials, we have already indicated that MyoIns treatment improves oocyte quality, by reducing, as shown by a growing number of trials, the number of degenerated and immature oocyte, in this way increasing the quality of embryos produced after fertilization of these cells [6–8,12].

The effect of MyoIns has also been tested in women not affected by PCOS but undergoing fertility treatment due to male factor or tubal factor. Beneficial effects highlighted in the treatment of PCOS women have also been confirmed in this setting, with particular reference to reduced amounts of FSH administered in order to obtain proper ovarian stimulation, furthermore, a promising trend in increasing clinical pregnancy has been highlighted [11]. Noteworthy, this result, confirmed in both PCOS patients and non-PCOS women, resembles the observation of Chiu et al. [4], who found that the amount of gonadotropin used for ovarian stimulation is reduced in women whose follicular fluid contains higher amounts of MyoIns.

Are there any differences in clinical efficacy between MyoIns and DCIns?

In a recently published study, Isabella and Raffone [61] administered increasing amounts of DCIns to insulin-resistant PCOS women undergoing IVF treatment, starting eight weeks prior to hormonal ovarian stimulation. The authors found that increasing DCIns dosage progressively worsens both ovarian response and oocyte/embryo quality, confirming previous observations [62]. This results has to be interpreted according to the following consideration: DCIns and MyoIns have different physiological roles since the former is crucial for glycogen synthesis while the latter increases cellular glucose uptake [20]. As already reported,

each tissue has its own MyoIns/DCIns ratio, reflecting specific functions of the two isomers, and high DCIns levels are present in glycogen storage tissues, such as fat, liver and muscle, whereas very low levels of DCI are characteristic of tissues with high glucose utilization, such as brain and heart [63]. According to this general tissue rule, the ovary would not require high doses of DCIns for its function.

In addition, it has also been reported that in the PCOS ovary, genes involved in glucose uptake are downregulated [64], with the hypothesis that a reduced energy metabolism is among the causes of poor oocyte quality in these patients [65]. These data are in line with the findings of Unfer et al. [9] who showed in a comparative study that MyoIns but not DCIns has an action at the ovarian level. It is thus likely that the beneficial effects of MyoIns on oocyte quality are linked to its double role in glucose cell uptake, which improves the ovary energy status, and in FSH signaling and induction of calcium release that allows proper germ cell maturation.

The negative effect of increasing doses of DCIns on the ovary, or DCIns paradox [66], has only recently drawn the attention of the scientific community on the importance of administering MyoIns and its stereoisomer in a physiological ratio, such as that observed in the blood serum [67], to restore proper tissue function. Indeed, in a recently published paper conducted on PCOS patients enrolled in an IVF program and divided in two groups according to their age ($\leq$35 and >35 years) [10], the combined therapy based on the physiological plasma ratio (40:1) has been compared to DCIns treatment alone. Results show that the combined therapy retains the beneficial effects of MyoIns treatment alone, outperforming the DCIns treatment, both at the level of ovarian response, with small differences between younger and older women, and at the level of oocyte and embryo quality, with no differences between the two groups [10].

Conclusions

Increasing evidence indicates that inositols exert a pivotal role namely in oocyte and spermatozoa development by itself or through its derivatives. The cellular content of inositol is represented almost entirely by MyoIns (>99%) and for the remaining part by a second stereoisomer, DCIns. MyoIns and DCIns have similar structures and differ in the stereochemistry of only one hydroxyl group, but despite this minor difference, their biological functions change dramatically [21,68,69]. Indeed, each tissue has its own MyoIns/DCIns ratio, reflecting specific functions played by the two isomers. Their respective proportions are actively maintained as MyoIns is enzymatically transformed into DCIns through a NAD, NADH-dependent epimerase, accordingly to tissue requirement. High DCIns levels are present in glycogen storage tissues, such as fat, liver and muscle, wheras very low levels of DCI are characteristic of tissues with high glucose utilization, such as brain and heart [63].

Indeed, an imbalance between MyoIns and DCIns leads to a reduction in insulin and FSH signaling, as observed in PCOS patients [51,66,71]. Indeed, MyoIns depletion induces a defect in glucose uptake. This in turn reduces glucose availability in the ovary for both oocytes and follicular cells. Although oocytes are characterized by high glucose consumption, by impairing sugar availability oocyte quality will be compromised [70].

Overall evidence from the literature analyzed by the Conference scientific Committee points out the beneficial effects of MyoIns treatment in ART, in particular at the level of ovarian response to exogenous gonadotropins as well as oocyte and embryo quality. In this regard, administration of MyoIns, alone or in combination with DCIns (in the physiological plasma ratio of 40:1), could be a predictive factor in improving ART outcomes.

Declaration of interest

The authors report no declarations of interest.

References

1. van Loendersloot LL, van Wely M, Limpens J, et al. Predictive factors in *in vitro* fertilization (IVF): a systematic review and meta-analysis. Hum Reprod Update 2010;16:577–89.
2. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. Hum Reprod Update 2010;17:34–45.
3. Revelli A, Delle Piane L, Casano S, et al. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reprod Biol Endocrinol 2009;7:40.
4. Chiu TT, Rogers MS, Law EL, et al. Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. Hum Reprod 2002;17:1591–6.
5. Pal L, Jindal S, Witt BR, Santoro N. Less is more... Increased gonadotropin use for ovarian stimulation adversely influences clinical pregnancy and live birth following IVF. Fertil Steril 2008; 89:1694–701.
6. Ciotta L, Stracquadanio M, Pagano I, et al. Effects of myo-inositol supplementation on oocyte's quality in PCOS patients: a double blind trial. Eur Rev Med Pharmacol Sci 2011;15:509–14.
7. Papaleo E, Unfer V, Baillargeon JP, et al. Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. Fertil Steril 2009;91:1750–4.
8. Unfer V, Raffone E, Rizzo P, Buffo S. 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. Gynecol Endocrinol 2011;27:857–61.
9. Unfer V, Carlomagno G, Rizzo P, et al. Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. Eur Rev Med Pharmacol Sci 2011;15:452–7.
10. Colazingari S, Treglia M, Najjar R, Bevilacqua A. 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. Arch Gynecol Obstet 2013;288:1405–11.
11. Lisi F, Carfagna P, Oliva MM, et al. Pretreatment with myo-inositol in non-polycystic ovary syndrome patients undergoing multiple follicular stimulation for IVF: a pilot study. Reprod Biol Endocrinol 2012;10:52.
12. Rizzo P, Raffone E, Benedetto V. 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. Eur Rev Med Pharmacol Sci 2010;14:555–61.
13. Chattopadhayay R, Ganesh A, Samanta J, et al. Effect of follicular fluid oxidative stress on meiotic spindle formation in infertile women with polycystic ovarian syndrome. Gynecol Obstet Invest 2010;69:197–202.
14. Berker B, Kaya C, Aytac R, Satiroglu H. Homocysteine concentrations in follicular fluid are associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction. Hum Reprod 2009;24:2293–302.
15. Condorelli RA, La Vignera S, Bellanca S, et al. Myoinositol: does it improve sperm mitochondrial function and sperm motility? Urology 2012;79:1290–5.
16. Chiu TT, Rogers MS, Briton-Jones C, Haines C. Effects of myo-inositol on the *in-vitro* maturation and subsequent development of mouse oocytes. Hum Reprod 2003;18:408–16.
17. Holm P, Booth PJ, Schmidt MH, et al. High bovine blastocyst development in a static *in vitro* production system using SOFaa medium supplemented with sodium citrate and myo-inositol with or without serum-proteins. Theriogenology 1999;52:683–700.
18. Warner SM, Conlon FV, Kane MT. Inositol transport in preimplantation rabbit embryos: effects of embryo stage, sodium, osmolality and metabolic inhibitors. Reproduction 2003;125:479–93. Erratum in: Reproduction 2005;129:128.
19. Colazingari S, Fiorenza MT, Carlomagno G, et al. Improvement of mouse embryo quality by myo-inositol supplementation of IVF media. J Assist Reprod Genet 2014;31:463–9.

20. Huang LC, Fonteles MC, Houston DB, et al. 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 1993; 132:652–7.
21. Sun TH, Heimark DB, Nguygen T, et al. Both myo-inositol to chiro-inositol epimerase activities and chiro-inositol to myo-inositol ratios are decreased in tissues of GK type 2 diabetic rats compared to Wistar controls. Biochem Biophys Res Commun 2002;293:1092–8.
22. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. Nature 2006;443:651–7.
23. Downes CP. The cellular functions of myo-inositol. Biochem Soc Trans 1989;17:259–68.
24. Downes CP, Macphee CH. myo-inositol metabolites as cellular signals. Eur J Biochem 1990;193:1–18.
25. Beemster P, Groenen P, Steegers-Theunissen R. Involvement of inositol in reproduction. Nutr Rev 2002;60:80–7.
26. Quirk Jr JG, Bleasdale JE. Myo-inositol homeostasis in the human fetus. Obstet Gynecol 1983;62:41–4.
27. Greene NDE, Copp AJ. Inositol prevents folate resistant neural tube defects in the mouse. Nat Med 1997;3:60–6.
28. Cavalli P, Copp AJ. Inositol and folate resistant neural tube defects. J Med Genet 2002;39:E5.
29. Lewin LM, Yannai Y, Melmed S, Weiss M. Myo-inositol in the reproductive tract of the female rat. Int J Biochem 1982;14:147–50.
30. Chiu TT, Tam PP. A correlation of the outcome of clinical *in vitro* fertilization with the inositol content and embryotrophic properties of human serum. J Assist Reprod Genet 1992;9:524–30.
31. Zacche MM, Caputo L, Filippis S, et al. Efficacy of myo-inositol in the treatment of cutaneous disorders in young women with polycystic ovary syndrome. Gynecol Endocrinol 2009;25:508–13.
32. Matsuda M, Tsutsumi K, Kanematsu T, et al. Involvement of phospholipase C-related inactive protein in the mouse reproductive system through the regulation of gonadotropin levels. Biol Reprod 2009;81:681–9.
33. 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: 441–51.
34. Lowther KM, Weitzman VN, Maier D, Mehlmann LM. Maturation, fertilization, and the structure and function of the endoplasmic reticulum in cryopreserved mouse oocytes. Biol Reprod 2009;81: 147–54.
35. Kwon HM, Yamauchi A, Uchida S, et al. Cloning of the cDNA for a Na^+/myo-inositol cotransporter, a hypertonicity stress protein. J Biol Chem 1992;267:6297–301.
36. Higgins BD, Kane MT. Inositol transport in mouse oocytes and preimplantation embryos: effects of mouse strain, embryo stage, sodium and the hexose transport inhibitor, phloridzin. Reproduction 2003;125:111–18.
37. Kane MT, Norris M, Harrison RAP. Uptake and incorporation of inositol by preimplantation mouse embryos. J Reprod Fertil 1992; 96:617–25.
38. Ajduk A, Ciemerych MA, Nixon V, et al. Fertilization differently affects the levels of cyclin B1 and M-phase promoting factor activity in maturing and metaphase II mouse oocytes. Reproduction 2008; 136:741–52.
39. Stachecki JJ, Armant DR. Transient release of calcium from inositol 1,4,5-trisphosphate-specific stores regulates mouse preimplantation development. Development 1996;122:2485–96.
40. Homburg R. Polycystic ovary syndrome – from gynaecological curiosity to multisystem endocrinopathy. Hum Reprod 1996;11: 29–39.
41. Ciampelli M, Fulghesu AM, Cucinelli F, et al. Impact of insulin and body mass index on metabolic and endocrine variables in polycystic ovary syndrome. Metabolism 1999;48:167–72.
42. Genazzani AD, Battaglia C, Malavasi B, et al. Metformin administration modulates and restores luteinizing hormone spontaneous episodic secretion and ovarian function in nonobese patients with polycystic ovary syndrome. Fertil Steril 2004;81:114–9.
43. Nestler JE, Strauss 3rd JF. Insulin as an effector of human ovarian and adrenal steroid metabolism. Endocrinol Metab Clin North Am 1991;20:807–23.
44. Marshall JC, Dunaif A. Should all women with PCOS be treated for insulin resistance? Fertil Steril 2012;97:18–22.
45. Costantino D, Minozzi G, Minozzi E, 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; 13:105–10.
46. Papaleo E, Unfer V, Baillargeon JP, et al. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. Gynecol Endocrinol 2007;23:700–3.
47. 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;11:347–54.
48. Nestler JE, Jakubowicz DJ, Reamer P, et al. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. N Engl J Med 1999;340:1314–20.
49. Iuorno MJ, Jacubowicz DJ, Baillargeon JP, et al. Effect of D-chiro-inositol in lean women with the polycystic ovary syndrome. Endocr Pract 2002;8:417–23.
50. Minozzi M, Costantino D, Guaraldi C, Unfer V. 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. Gynecol Endocrinol 2011;27:920–4.
51. Unfer V, Carlomagno G, Papaleo E, et al. Hyperinsulinemia alters myoinositol to D-chiroinositol ratio in the follicular fluid of patients with PCOS. Reprod Sci 2014;21:854–8. [Epub ahead of print]. PubMed PMID:24501149.
52. Eisenberg Jr F, Bolden AH. Reproductive tract as site of synthesis and secretion of inositol in the male rat. Nature 1964;202: 599–600.
53. Chauvin TR, Griswold MD. Characterization of the expression and regulation of genes necessary for myo-inositol biosynthesis and transport in the seminiferous epithelium. Biol Reprod 2004;70: 744–51.
54. Hinton BT, White RW, Setchell BP. Concentrations of myo-inositol in the luminal fluid of the mammalian testis and epididymis. J Reprod Fertil 1980;58:395–9.
55. Cryns K, Shamir A, Van Acker N, et al. IMPA1 is essential for embryonic development and lithium-like pilocarpine sensitivity. Neuropsychopharmacol 2008;33:674–84.
56. Martinez-Heredia J, de Mateo S, Vidal-Taboada JM, et al. Identification of proteomic differences in asthenozoospermic sperm samples. Hum Reprod 2008;23:783–91.
57. Colone M, Marelli G, Unfer V, et al. Inositol activity in oligoasthenoteratospermia – an *in vitro* study. Eur Rev Med Pharmacol Sci 2010;14:891–6.
58. Condorelli RA, La Vignera S, Di Bari F, et al. Effects of myoinositol on sperm mitochondrial function *in-vitro*. Eur Rev Med Pharmacol Sci 2011;15:129–34.
59. Marchetti C, Jouy N, Leroy-Martin B, et al. Comparison of four fluorochromes for the detection of the inner mitochondrial membrane potential in human spermatozoa and their correlation with sperm motility. Hum Reprod 2004;19:2267–76.
60. Marchetti P, Ballot C, Jouy N, et al. Influence of mitochondrial membrane potential of spermatozoa on *in vitro* fertilisation outcome. Andrologia 2012;44:136–41.
61. Isabella R, Raffone E. Does ovary need D-chiro-inositol? J Ovarian Res 2012;5:14.
62. Cheang KI, Baillargeon JP, Essah PA, et al. Insulin-stimulated release of D-chiro-inositol-containing inositol phosphoglycan mediator correlates with insulin sensitivity in women with polycystic ovary syndrome. Metabolism 2008;57:1390–7.
63. Larner J. D-chiro-inositol – its functional role in insulin action and its deficit in insulin resistance. Int J Exp Diabetes Res 2002;3:47–60.
64. Ma X, Fan L, Meng Y, et al. Proteomic analysis of human ovaries from normal and polycystic ovarian syndrome. Mol Hum Reprod 2007;13:527–35.
65. Arya BK, Haq AU, Chaudhury K. Oocyte quality reflected by follicular fluid analysis in poly cystic ovary syndrome (PCOS): a hypothesis based on intermediates of energy metabolism. Med Hypotheses 2012;78:475–8.
66. Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. Fertil Steril 2011;95:2515–6.
67. Nordio M, Proietti E. The combined therapy with myo-inositol and D-Chiro-inositol reduces the risk of metabolic disease in PCOS overweight patients compared to myo-inositol supplementation alone. Eur Rev Med Pharmacol Sci 2012;16:575–81.

68. Larner J, Huang LC, Tang G, et al. Insulin mediators: structure and formation. Cold Spring Harb Symp Quant Biol 1988;53:965–71.
69. Majumder AL, Biswas BB. Biology of inositols and phoshoinositides. Subcellular Biochemistry. The Netherlands; Springer; 2006:2039.
70. Chaudhary K, Babu KN, Joshi VN, et al. NMR-based metabolomics reveals differently expressed metabolites in follicular fluid of PCOS women: potential biomarkers for good quality oocyte? Hum Reprod 2011;26:i226–46.
71. Heimark D1, 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. Endocr J 2014;61:111–17.