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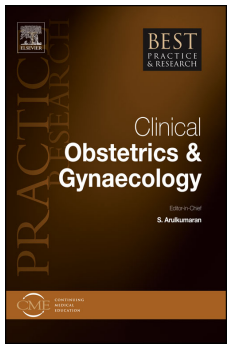

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Physiological role and clinical utility of Inositols in Polycystic Ovary Syndrome

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Abstract

During the last decades, a substantial body of research has been focused on the role of the two major inositol stereoisomers, myo-inositol and D-chiro-inositol, both second messengers of insulin, in insulin-dependent processes, including polycystic ovary syndrome (PCOS). MyoIns has been showed to affect different pathways at both the ovarian and non-ovarian level. On the contrary, D-chiro-inositol alone is unable to exert valuable improvements in ovary cell functions, as its beneficial effects are mainly limited to non-ovarian tissue in which it may significantly inhibit the negative cellular consequences of hyperinsulinemia. However, both inositol isomers can be positively associated in the management of PCOS patients in a ratio corresponding to their physiological plasma ratio (40:1). This appears to exert a synergistic effect according to a multi-targeted design. In this respect, new fundamental insights into biological mechanisms displayed by inositols, as well as clinical trials based on the myo-inositol + D-chiro-inositol formulations, have already provided encouraging results.

Key words: Polycystic Ovary Syndrome, insulin, inositol, myoinositol, D-chiroinositol.

Biochemistry and metabolism of Inositols

During recent years, studies on inositols (Ins) and its phosphate derivatives such as, for example myo-inositol-1-phosphate, myo-inositol-bisphosphate, inositol pentakiphosphate, have gained momentum [1].

Inositol and its derivatives are natural compounds abundant in fruits and some vegetables, representing a family of nine hexahydroxycyclohexane stereoisomers with general formula $C_6H_{12}O_6$. Inositols are chemically very stable polar molecules with versatile properties. Originally isolated by J.J. Scherer in 1850 from muscle tissues, they later appeared to be broadly distributed in mammalian tissues and cells, where they perform important biologic functions. This is particularly true for at least six Ins isomers, myo-inositol, scyllo-inositol, epi-inositol, neo-inositol, D-chiro-inositol and muco-inositol [2].

Even though inositol has been deemed for a while to be an "inactive" molecule [3], current evidence demonstrates it plays significant biological properties by itself. Inositol and Ins derived molecules, besides their "traditional" function as mineral "stores", participate in regulating a plethora of cellular pathways. Indeed, cell signaling via inositol and inositol phosphates, in particular via the second messenger myo-inositol 1,4,5-trisphosphate, comprises a huge field of biology, this way participating in the regulation of a plethora of cellular pathways: insulin signal transduction, Ca^{2+} flow, cytoskeletal proteins assembly, lipid metabolism, modulation of serotoninergic pathways, cell growth and differentiation, oocyte maturation and fertility [4].

Moreover, some inositol isomers have been proven to be medically relevant: scyllo-Ins in neurodegenerative diseases, D-chiroIns in diabetes and myoIns in cancer, metabolic syndrome, polycystic ovary syndrome (PCOS)[5]. It is therefore appropriate to consider looking at the roles and applications of these "old" simple molecules as "new" pharmacological, pleiotropic agents. Therefore, scientific literature on inositol and its diverse phosphate derivatives is continuously updated in order to cover the widespread array of released studies [6]. Inositol use, both as nutraceutical-based preventive strategy as well as pharmacological support in medical treatments, has been proposed in many diseases, including psychiatric/neurological illnesses [7], infertility, diabetes, inflammatory processes [8] and cancer [9].

Myo-inositol and D-chiro-inositol

Myo-inositol (myoIns) is the most abundant form of Ins both in nature and in mammalian cells, with up to 99% of the overall Ins amount. The remaining 1% is represented by the other stereoisomer, D-chiro-inositol (D-chiroIns). Although having different metabolic functions, both of these molecules are mediators of insulin action inside the cell [10]. MyoIns is converted into D-chiroIns by a NAD/NADH epimerase, an enzyme the expression of which is tissue-specific and insulin dependent. For these reasons, activity of this enzyme strongly influences different concentration ratios observed between the two molecules in hepatic or adipose cells, or muscle fibers [11].

MyoIns has defined roles in both somatic and germ cells, as a precursor of phosphoinositides, membrane components, signaling molecules (reviewed by [12, 13]), and as hyperosmotic stress protectant [14].

Inside cells, myoIns is present both as free form and as component of membrane phosphoinositides. Phosphatidyl-myo-inositol is the precursor of phosphatidyl-inositol phosphate and phosphatidyl-inositol bisphosphate (PIP_2), molecules with important physiological roles [15]. Hydrolysis of PIP_2 by phospholipase C (PLC) produces inositol trisphosphate (Ins-1,4,5P_3, InsP_3), which acts as second messenger regulating the activities of several hormones such as FSH, TSH, and insulin [15,16]. 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, transport and break-down [17], and the regulation of cell proliferation [18,19], relevant during development in all its phases [20–23]: pre- as well as post-implantation embryogenesis and, in the adult, oogenesis and spermatogenesis.

In mammals, a tissue specific epimerase converts myoIns into D-chiroIns. This reaction is induced by insulin [17] and is particularly sustained in hepatic and muscle cells where it is involved in glycogen synthesis. Differences in myoIns/D-chiroIns distribution are responsible for the distinct functions the two isomers play in the tissues.

D-chiroIns is considered to be a relevant component of the insulin-signaling pathway, participating along with galactosamine in a wide array of molecular mechanisms, including stimulation of pyruvate dehydrogenase phosphatase, protein phosphatase 2C, inositol-phosphate glycan (IPG) [24] that altogether seem to dump the consequences of deregulated glucose metabolism. This role is, however, still controversial, since serum D-chiroIns levels are increased in insulin-resistant women with pre-eclampsia, and elevated D-chiroIns levels are suspected to contribute to insulin resistance [25]. In rats D-chiroIns has to be considered an essential nutrient, as it cannot be synthesized endogenously nor be produced from myo-Ins [26].

Yet, D-chiroIns is required to ensure proper development. Indeed, mice genetically engineered to develop folate-resistant neural-tube defects in utero can be more effectively treated with D-chiroIns than with Myo-Ins [23,27] In addition, D-chiroIns is able to prevent and reverse endothelial dysfunction or bone architectural defects in both rats and rabbits [28,29].

Inositol in Gynaecology and Fertility

During the last decades, gathered data hint for a significant role exerted by Ins in several pathological conditions belonging to gynaecology, as well as in fertility related processes, including oocyte maturation and sexual structure differentiation. These pieces of evidence advocate for a relevant role sustained by Ins and its metabolites in human reproduction, as claimed by Beemster et al. [20] seminal paper.

Inositol and reproductive function: a role in oogenesis and embryogenesis

Inositols, and myoIns in particular, represent important molecules for early development (reviewed by [20]), its serum concentrations in foetuses and newborn infants being several folds higher than in adults [21]. Its administration during pregnancy reduces the risk of gestational diabetes in humans [30,31] and the occurrence of developmental defects in the foetuses of mutant mice [22]. MyoIns also plays a positive role in reproduction: its concentration in mammalian female reproductive tracts [32] is substantially higher than that of blood serum, suggesting its positive influence on fertility; its levels in the follicular fluid and in blood serum are positively correlated with oocyte quality and pregnancy outcome in humans [32,33]; administration of myoIns to women before the onset of hormonal stimulation in IVF cycles increases oocyte [34-36] and embryo quality [34,37,38], and reduces the amount of FSH and days needed for proper stimulation, parameters directly linked to the chance of pregnancy [39].

At the ovarian level, myoIns appears involved in different functions, being essential to ensure proper oocyte maturation. MyoIns action is related to the role played by $InsP_3$ on the modulation of intracellular calcium ion concentration in response to LH and FSH action [40,41]. In oocytes, this mechanism, acting through interaction with specific receptors ($InsP_3$-R1) [42], plays a central role in the maturation process [43]. Culture medium supplementation with myoIns has been shown to increase meiotic maturation of mouse oocytes with the production of fertile eggs, while the depletion of intracellular stores of myoIns desensitizes inositol-dependent transduction pathways, reducing levels of InsP3 and the proper release of calcium with a negative impact on oocyte maturation [44]. When oocytes matured in the presence of myoIns were fertilized *in vitro* and transferred to foster mothers, the implantation rate and postimplantation viability of the resulting embryos was also increased [44].

Experiments on farming species suggest a role for myoIns in mammalian preimplantation development, since myoIns supplementation of culture media improves rabbit and bovine blastocyst formation, expansion and hatching [45,46], and allows development to term of healthy animals [45].

Active transport systems allow the uptake of myoIns into mammalian cells, including oocytes and preimplantation embryos. In the mouse, activity of at least two different membrane transporters [47,48] is responsible of a progressive increase in myoIns uptake during early development, between the one-cell stage and the blastocyst stage [49]. MyoIns is then rapidly incorporated into phosphoinositides [49]. In the zygote, concentration oscillations of calcium ions, induced by PLC-dependent $InsP_3$ production, have key roles, from egg activation at fertilization [50] to blastomere divisions [51]. The inclusion of myoIns in human embryo culture media has recently been shown to allow *in vitro* produced and cultured mouse embryos to complete preimplantation development and develop to the expanded blastocyst stage at rates similar to *in vivo* developed embryos [52]. This led us to hypothesize that inclusion of this molecule in embryo culture media would produce an increase in the number of high quality embryos produced in human IVF cycles [52].

Inositol in gynaecology diseases

The last decade has witnessed an increasing body of data concerning the usefulness of inositols in the clinical management of PCOS. The polycystic ovary syndrome, first described by Stein and Leventhal, is one of the most common female endocrine disorders [53].

Despite the controversy about differences among clinical features of PCOS [54], it is now widely recognized that insulin resistance (IR) plays a significant pathogenetic role in a large sub-set of patients [55]. Insulin resistance in PCOS women is primarily ascribed to defects in post-binding signaling [56], hence affecting several downstream targets, including modulation of steroidogenic pathways. Insulin directly prompts the ovary theca cells to enhance the synthesis and release of androgens both directly and indirectly, by modulation of carbohydrate levels. In turn, high glucose levels inhibit the hepatic synthesis of sex hormone-binding globulin, thereby causing a consequent increase of circulating free-active androgens [57]. Since the relationships among disturbances in insulin signaling and PCOS were established, several antidiabetic, insulin sensitizing drugs, including Metformin and Thiazolidinediones, have been

investigated as effective adjunct in treating PCOS patients. In particular, Metformin reduces glucose serum levels, down-regulates ovarian androgen production and decreases circulating androgen levels [58].

However, while Metformin has shown to counteract hyperandrogenism in the short term in obese, insulin-resistant PCOS women [59], controversial results have been published about Metformin efficacy in non-obese non-insulin-resistant patients [60]. Additionally, Metformin has a detrimental effect on follicle number and quality [61].

Moreover, both Metformin and Thiazolidinediones are burdened by relevant adverse events including gastrointestinal symptoms, metabolic complications [62], fluid retention, body weight increase, coronary artery disease, bladder cancer [63]. Yet, it is intriguing that benefits obtained in Metformin-treated PCOS women have been ascribed to a secondary increase in availability of inositol phosphoglycans triggered by Metformin administration [64], thus providing support to the hypothesis that insulin-signaling pathways require Ins.

Indeed, when insulin binds to its receptor, two distinct inositol-phosphoglycans (IPG), incorporating either myoIns or D-chiroIns (D-chiroIns-IPG and myoIns-IPG), are released by hydrolysis of glycosyl-phosphatidylinositol lipids on the outer side of cell membrane. In turn, IPG affect intracellular metabolic processes by activating key enzymes controlling the oxidative and non-oxidative glucose metabolism [65]. Even though some differences have been recorded, both D-chiroIns- and myoIns-containing IPG significantly reduce IR and improve glucose metabolism [66].

Women affected by PCOS show reduced serum levels of D-chiroIns and increased urinary loss of D-chiroIns-IPG [67]. This observation fostered further investigations that eventually ended up in demonstrating that PCOS patients experience a severe deregulation of inositol metabolism, thus enabling the establishment of a clear mechanistic link between IR and inositol deficiency in PCOS patients [68]. Early clinical studies were aimed at verify the D-chiroIns usefulness in PCOS management. Impressive results were indeed obtained, as PCOS patients treated with low doses of D-chiroIns (i.e 1.2 g/daily) showed reduced levels of lipid biomarkers, increased insulin sensitivity, decreased serum androgen levels and higher ovulation frequency [69,70].

Those effects have been mainly ascribed to a D-chiroIns systemic activity, able to counteract the main consequences of the metabolic syndrome that is frequently associated with PCOS [71]. However, when administered at higher doses, D-chiroIns

seems to exert negative effects on ovarian tissues. Indeed, subsequent clinical trials performed with D-chiroIns doses of 2,4 g/daily, were unable to confirm previous positive results on PCOS women, eventually suggesting that D-chiroIns may paradoxically worsen the ovarian response in non insulin-dependent PCOS patients, even if D-chiroIns was effective in normalizing IR parameters [72,73]. These results showed that counteracting IR might not be sufficient to reverse clinical features of PCOS. Indeed, similar disappointing results have been recorded by treating non-insulin-resistant PCOS women with Metformin. The antidiabetic drug improves PCOS clinical signs only in a fraction of PCOS patients, but worsens their oocyte quality [74], thus highlighting the idea that IR is likely not to be the main causative factor in PCOS pathogenesis. In addition, some data indicate that D-chiroIns may exert detrimental effects on ovary tissues. Release of high levels of D-chiroIns-IPG under insulin stimulation promotes testosterone biosynthesis from ovarian theca cells, thus leading to increased serum androgen levels [75].

Additionally, D-chiroIns may significantly hinder myoIns uptake in mammalian cells, leading to an imbalance in their respective ratio within the ovary [76]. As already discussed, myoIns is in fact the most abundant inositol isomer within the ovary, accounting for about 99% of all inositol [77], and is converted to D-chiroIns through an insulin-induced, NAD-dependent epimerase. A decreased epimerase activity has been observed in human muscle tissue of type 2 diabetics [78] and in other non-ovarian tissues in IR patients [79]. Yet, as the ovarian tissue never develops insulin insensitivity, higher circulating insulin levels are likely to induce a prominent and paradoxical increase in D-chiroIns concentration within the ovary. In turn, increased conversion from myoIns to D-chiroIns would dramatically reduce myoIns levels within ovary cells. Specifically, a significant increase in epimerase activity in theca cells of ovaries of PCOS women has been found to be associated with a dramatic reduction in myoIns/ D-chiroIns ratio [80]. Indeed, a significant decrease in the myoIns/ D-chiroIns ratio has been documented in the follicular fluid of PCOS patients [34]. Consequently, any further D-chiroIns increase is likely detrimental to ovarian function, thus providing a mechanistic explanation to the so-called “D-chiroIns ovarian paradox” [81]. Furthermore, D-chiroIns has been proven to be largely ineffective in non-hyperinsulinemic PCOS women since the majority of these patients does not respond to D-chiroIns therapy [81].

On the contrary, usefulness of myoIns supplementation in PCOS has been assessed by several reports. In mice, myoIns improves glucose tolerance by increasing insulin sensitivity and potentiates insulin activity [83]. Namely, myoIns promotes the translocation of glucose transporter 4 to the plasma membrane, therefore lowering plasma glucose and insulin levels [84].

MyoIns supplementation in PCOS patients counteracts the main features of the associated metabolic syndrome and improves several ovarian functions: oocyte quality, frequency of ovulation, increased pregnancy rate, reduced number of FSH treatment required to trigger ovulation. These results have been assessed by both pilot and randomized studies [38,85-88].

It is worth to outline that the frequency of both ovulation and pregnancy rate were both raised by myoIns treatment [35,89]. It thus appears that myoIns improves both ovarian function and systemic features associated with PCOS: deregulation of glucose metabolism, clinical signs of hyperandrogenism, lipid metabolism [40,90].

Facchinetti et al. [91] reviewed and analyzed six Randomized Controlled Trials focused on myoIns supplementation in PCOS patients and provided strong evidence for a higher myoIns effectiveness (with a dosage of 2-4 g/day for 12-16 weeks) with respect to conventional therapy or to treatments based on D-chiroIns alone. Yet, a potential bias is still represented by the fact that other studies lack proper randomization or are flawed by statistical weakness [92]. Moreover, we still await a compelling investigation on inositol-based mechanisms within the ovary, even if evidence is continuously rising.

Functional difference among D-chiroIns and myoIns may help in understanding why different myoIns/D-chiroIns ratios are actively preserved in fat, muscle, liver and ovarian tissues, through a tuned regulation of epimerase activity. While D-chiroIns effects are mainly restricted to insulin signaling transduction, myoIns has demonstrated to exert other noticeable activities (see Fig. 1), by influencing cytoskeleton remodeling [Bizzarri M, unpublished observations], steroidogenesis and oocyte maturation [93]. Furthermore, myoIns participates in modulating LH/FSH activity [41] and GnRH agonist-mediated LH inhibition [94]. Both aromatase and 3α-hydroxysteroid dehydrogenase activities are modulated by D-chiroIns and myoIns in a subtle diverse fashion [95,96]. Moreover, a relative shortage of myoIns has been hypothesized from an epidemiological point of view, to impair fertility and ovary function [97]. Namely, myoIns has been deemed to significantly modulate

steroidogenesis by acting through an insulin-independent pathway that involves cytoskeleton rearrangements [98]. As of now, the rationale on which the inositol-based treatment of PCOS mainly relies on the insulin mimicking effect of IPG. However, it is currently recognized that during the development of PCOS, hyperinsulinism or insulin resistance act as a second hit, worsening the follicular arrest either through amplification of the intra-ovarian hyperandrogenism or through dysregulation of granulosa cells [99]. Given that intra-ovarian deregulation of androgens is currently thought as a main culprit for follicular arrest in PCOS, the insulin-independent anti-androgenic effect displayed by myoIns deserves to be investigated thoroughly.

<insert Fig. 1 near here>

Selectivity and diversity of inositol isomers activity eventually provided the rationale for a new, different therapeutic approach [100]. Accordingly, a proper treatment should integrate the positive effects exerted by both inositol isoforms, combined according to the physiological myoIns/D-chiroIns plasma ratio of 40:1. A few clinical trials in which PCOS women have been treated with a combination of myoIns + D-chiroIns (40:1)[91] have lead to promising results in support of that rationale. Significant better outcomes were observed in the myoIns + D-chiroIns patients when compared with women treated with D-chiroIns (500 mg/daily) alone [37]. Subsequent studies revealed that several PCOS metabolic features (diastolic blood pressure, fasting glucose, fasting insulin, HOMA index, triglycerides, HDL and LDL cholesterol levels and both insulin and glucose AUCs) altogether with ovulation rate were significantly improved in myoIns + D-chiroIns treated patients [101,102].
Again, additional studies are warranted to precisely determine the appropriate proportion in which myoIns and D-chiroIns should be mixed to trigger a maximal beneficial effect. This implies that detail pharmacodynamics analysis and careful randomisation trials are needed to properly address such issues.

Conclusions and future perspectives

Although D-chiroIns and myoIns have different functions, very often their roles have been confused, while the meaning of several observations still needs to be interpreted under a more rigorous physiological framework. To clarify this issue, the 2013

International Consensus Conference on Myo-Ins and D-chiroIns in Obstetrics and Gynaecology gathered opinion leaders in all fields related to this area of research. They examined seminal experimental papers and randomized clinical trials reporting the role and the use of inositol(s) in clinical practice, answering a number of questions concerning the use of the two stereoisomers in gynaecology [103].

Yet, there are still numerous questions awaiting response about the mechanism of action displayed by inositols, chiefly regarding their role in glucose metabolism and insulin-dependent signaling [104]. Studies on the effects displayed by Ins during oocyte maturation, embryonic development and organogenesis are still in their infancy and need to be urgently implemented. Additionally, emerging evidence suggest that D-chiroIns and myoIns exert within the ovary opposite effects depending on their concentrations, on different targets of gynaecological interest. Both isomers seem to positively affect glucose metabolism in several insulin-sensitive tissues, while in the ovary normal homeostasis require an appropriate myoIns/D-chiroIns ratio, given that high D-chiroIns levels showed to be detrimental. These data provided the rationale in attempting to associate both Ins isomers according to their physiological respective levels. Yet, this assumption still awaits further confirmation. Namely, it is worth to outline some outstanding issues that should be addressed in order to achieve further progress on the field. First, the metabolism of Ins and the dynamic interplay among its diverse phosphate-derivatives must be thoroughly investigated. This metabolomic approach shall enable the understanding of the relevant Ins-based messengers and their involvement in specific molecular pathways. Second, it would be of great relevance to fully elucidate the Ins participation in non insulin-related mechanisms. MyoIns and its derivatives participate in modulating cytoskeleton and this in turn significantly influences steroidogenesis processes. It would therefore be tempting to speculate that androgen release as well could be regulated by Ins through cytoskeleton-related pathways. Given that hyper-androgenism is deemed a key pathogenetic mechanism in PCOS, such aspect deserve to be investigated in dept. Third, our knowledge of the selective activity of epimerase within different tissue should be studies appropriately to grasp how the myoIns/D-chiroIns ratio is finely tuned to fulfil diverse tasks. Eventually, the participation of Ins in endocrine signaling, with special reference to LH and FSH, still needs to be addressed at the molecular level.

Thereby, we believe that focusing on that question will significantly advance our comprehension of the role Ins displays in gynaecological illness.

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Authors declare no conflicts of interests.

Practice points

- myoIns + D-chiroIns can be positively associated in the management of PCOS patients in a ratio corresponding to their physiological plasma ratio (40:1)
- the combined myoIns + D-chiroIns administration is safe and provides better results than conventional treatment of PCOS women at both the systemic and ovarian level
- myoIns is required for proper oocyte maturation
- the combined myoIns + D-chiroIns administration is effective even in non insulin-resistant women

Research agenda

- to precisely determine the appropriate proportion in which myoIns and D-chiroIns should be administered to produce maximal treatment efficacy.
- to complete analysis of myoIns effects during embryonic development, organogenesis and after birth in a mouse model.
- to investigate by a metabolomic approach the involvement of relevant Ins-based second messengers in specific molecular pathways
- to understand molecular mechanisms that allow Ins intervention in endocrine signaling, with specific reference to LH and FSH

References

1. Thomas MP, Mills SJ, Potter BV. The "Other" Inositols and Their Phosphates: Synthesis, Biology, and Medicine (with Recent Advances in myo-Inositol Chemistry). *Angew Chem Int Ed Engl* 2015; **54**: 2–39.

2. Bizzarri M, Carlomagno G. Inositol: history of an effective therapy for Polycystic Ovary Syndrome. *Eur Rev Med Pharmacol Sci* 2014; **18**(13):1896-903.

3. Hoglan FA, Bartow E. Inactive inositol and its derivatives. *Ind Eng Chem* 1939; **31**(6), 749–50.

* 4. Holub BJ. Metabolism and function of myo-inositol and inositol phospholipids. *Annu Rev Nutr* 1986; **6**: 563-97.

5. Majumder AL, Biswas BB Eds. Biology of inositols and Phosphoinositides. 2010; Springer, Berlin.

6. Falasca M Ed. Phosphoinositides and disease. 2012; Springer, Berlin.

7. Palatnik A, Frolov K, Fux M, Benjamin J. Doubleblind, controlled, crossover trial of inositol versus fluvoxamine for the treatment of panic disorder. *J Clin Psychopharm* 2001; **21**: 335-9.

8. Pasta V, Gullo G, Giuliani A., Harrath AH, Alwasel SH, Tartaglia F, Cucina A, Bizzarri M. An association of boswellia, betaine and myo-inositol (eumastós®) in the treatment of mammographic breast density: a randomized, double-bind study. *Eur Rev Med Pharmacol Sci* 2015; **19**(22): 4419-26.

9. Vucenik I, Shamsuddin AM. Cancer inhibition by inositol hexaphosphate (IP6) and inositol: from laboratory to clinic. *J Nutr* 2003 Nov; **133**(11 Suppl 1): 3778S-84S.

10. Huang LC, Fonteles MC, Houston DB, Zhang C, Larner J. Chiroinositol deficiency and insulin resistance. III. Acute glycogenic and hypo- glycemic effects of two inositol phosphoglycan insulin mediators in normal and streptozotocin-diabetic rats in vivo. *Endocrinology* 1993; **132**: 652–7.

11. Sun TH, Heimark DB, Nguygen T, Nadler JL, Larner J. 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.

12. Berridge MJ. Inositol trisphosphate and calcium oscillations. *Adv Second Messenger Phosphoprotein Res* 1992; **26**: 211–23.

13. Downes CP, Macphee CH. Myo-inositol metabolites as cellular signals. *Eur J Biochem* 1990; **193**(1): 1–18.

14. Strange K, Morrison R, Heilig CW, DiPietro S, Gullans SR. Upregulation of inositol transport mediates inositol accumulation in hyperosmolar brain cells. *Am J Physiol* 1991; **260**(4 Pt 1): C784–90.

15. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. *Nature* 2006; **443**: 651–7.

16. Thomas RM, Nechamen CA, Mazurkiewicz JE, Ulloa-Aguirre A, Dias JA. The adapter protein APPL1 links FSH receptor to inositol 1,4,5-trisphosphate production and is implicated in intracellular Ca(2+) mobilization. *Endocrinology* 2011; **152**: 1691–701.

17. 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. *Endocr J* 2014; **61**: 111–7.

18. Downes CP. The cellular functions of myo-inositol. *Biochem Soc Trans* 1989; **17**: 259–68.

19. Downes CP, Macphee CH. Myo-inositol metabolites as cellular signals. *Eur J Biochem* 1990; **193**: 1–18.

* 20. Beemster P, Groenen P, Steegers-Theunissen R. Involvement of inositol in reproduction. *Nutr Rev* 2002; **60**: 80–7.

21. Quirk Jr JG, Bleasdale JE. Myo-inositol homeostasis in the human fetus. *Obstet Gynecol* 1983; **62**: 41–4.

22. Greene NDE, Copp AJ. Inositol prevents folate resistant neural tube defects in the mouse. *Nat Med* 1997; **3**: 60–6.

23. Cavalli P, Copp AJ. Inositol and folate resistant neural tube defects. *J Med Genet* 2002; **39**: E5.

24. Larner J. D-chiro-inositol--its functional role in insulin action and its deficit in insulin resistance. *Int J Exp Diabetes Res* 2002; **3**(1): 47-60.

25. Scioscia M, Nigro M, Montagnani M. The putative metabolic role of d-chiro inositol phosphoglycan in human pregnancy and preeclampsia. *J Reprod Immunol* 2014; **101-102**: 140-7.

26. Lin X, Ma L, Gopalan C, Ostlund RE. d-chiro-Inositol is absorbed but not synthesized in rodents. *Br J Nutr* 2009; **102**(10): 1426-34.

27. Cogram P, Tesh S, Tesh J, Wade A, Allan G, Greene ND, Copp AJ. D-chiro-inositol is more effective than myo-inositol in preventing folate-resistant mouse neural tube defects. *Hum Reprod* 2002; **17**(9): 2451-8.

28. Nascimento NR, Lessa LM, Kerntopf MR, Sousa CM, Alves RS, Queiroz MG, Price J, Heimark DB, Larner J, Du X, Brownlee M, Gow A, Davis C, Fonteles MC. Inositols prevent and reverse endothelial dysfunction in diabetic rat and rabbit vasculature metabolically and by scavenging superoxide. *Proc Natl Acad Sci USA* 2006; **103**(1): 218-23.

29. Yu J, Choi S, Park ES, Shin B, Yu J, Lee SH, Takami M, Kang JS, Meong H, Rho J. D-chiro-inositol negatively regulates the formation of multinucleated osteoclasts by down-regulating NFATc1. *J Clin Immunol* 2012; **32**(6): 1360-71.

30. D'Anna R, Di Benedetto V, Rizzo P, Raffone E, Interdonato ML, Corrado F, Di Benedetto A. Myo-inositol may prevent gestational diabetes in PCOS women. *Gynecol Endocrinol* 2012; **28**(6): 440–2.

31. D'Anna R, Scilipoti A, Giordano D, Caruso C, Cannata ML, Interdonato ML, Corrado F, Di Benedetto A. Myo-Inositol supplementation and onset of gestational diabetes mellitus in pregnant women with a family history of type 2 diabetes: a prospective, randomized, placebo-controlled study. *Diabetes Care* 2013; **36**(4): 854–7.

* 32. 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**(6): 524–30.

33. Chiu TT, Rogers MS, Law EL, Briton-Jones CM, Cheung LP, Haines CJ. Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. *Hum Reprod* 2002; **17**: 1591–6.

34. Ciotta L, Stracquadanio M, Pagano I, Carbonaro A, Palumbo M, Gulino F. Effects of Myo-Inositol supplementation on oocyte's quality in PCOS patients: a double blind trial. *Eur Rev Med Pharmacol Sci* 2011; **15**(4): 509–14.

35. Papaleo E, Unfer V, Baillargeon JP, Fusi F, Occhi F, De Santis L. Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. *Fertil Steril* 2009; **91**(5): 1750–4.

36. Unfer V, Carlomagno G, Rizzo P, Raffone E, Roseff S. 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**(4): 452–7.

* 37. 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**(6): 1405–11.

38. Lisi F, Carfagna P, Oliva MM, Rago R, Lisi R, Poverini R, Manna C, Vaquero E, Caserta D, Raparelli V, Marci R, Moscarini M. Pretreatment with myo-inositol in non polycystic ovary syndrome patients undergoing multiple follicular stimulation for IVF: a pilot study. *Reprod Biol Endocrinol* 2012; **23**(10): 52.

39. 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**(6): 1694–701.

40. Zacchè MM, Caputo L, Filippis S, Zacchè G, Dindelli M, Ferrari A. Efficacy of myo-inositol in the treatment of cutaneous disorders in young women with polycystic ovary syndrome. *Gynecol Endocrinol* 2009; **25**: 508–13.

41. Matsuda M, Tsutsumi K, Kanematsu T, Fukami K, Terada Y, Takenawa T, Nakayama KI, Hirata M. Involvement of phospholipase C-related inactive protein in the mouse reproductive system through the regulation of gonadotropin levels. *Biol Reprod* 2009; **81**: 681–9.

42. 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.

43. 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.

44. 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.

45. Holm P, Booth PJ, Schmidt MH, Greve T, Callesen H. 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**(4): 683–700.

46. Warner SM, Conlon FV, Kane MT. Inositol transport in preimplantation rabbit embryos: effects of embryo stage, sodium, osmolality and metabolic inhibitors. *Reproduction* 2003; **125**(4): 479–93. Erratum in: *Reproduction* 2005; **129**(1): 128.

47. Kwon HM, Yamauchi A, Uchida S, Preston AS, Garcia-Perez A, Burg MB, Handler JS. Cloning of the cDNA for a Na+/myo-inositol cotransporter, a hypertonicity stress protein. *J Biol Chem* 1992; **267**: 6297–301.

48. 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.

49. Kane MT, Norris M, Harrison RAP. Uptake and incorporation of inositol by preimplantation mouse embryos. *J Reprod Fertil* 1992; **96**: 617–25.

50. Ajduk A, Ciemerych MA, Nixon V, Swann K, Maleszewski M. 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.

51. 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.

* 52. Colazingari S, Fiorenza MT, Carlomagno G, Najjar R, Bevilacqua A. Improvement of mouse embryo quality by myo-inositol supplementation of IVF media. *J Assist Reprod Genet* 2014; **31**: 463–9.

53. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010; **25**: 544-51.

54. The 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). *Hum Reprod* 2004; **19**: 41–7.

55. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 1989; **38**(9): 1165–74.

56. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* 1997; **18**: 774–800.

57. Toprak S, Yonem A, Cakir B, Güler S, Azal O, Ozata M, Corakçi A. Insulin resistance in nonobese patients with polycystic ovary syndrome. *Horm Res* 2001; **55**: 65–70.

58. Palomba S, Falbo A, Zullo F, Orio F Jr. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. *Endocr Rev* 2009; **30**: 1-50.

59. Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L. Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. *Fertil Steril* 2000; **73**: 1149-54.

60. Trolle B, Flyvbjerg A, Kesmodel U, Lauszus FF. Efficacy of metformin in obese and non-obese women with polycystic ovary syndrome: a randomized, double-blinded, placebo-controlled crossover trial. *Hum Reprod* 2007; **22**: 2967-73.

61. Palomba S, Falbo A, Di Cello A, Cappiello F, Tolino A, Zullo F. Does metformin affect the ovarian response to gonadotropins for in vitro fertilization treatment in patients with polycystic ovary syndrome and reduced ovarian reserve? A randomized controlledtrial. *Fertil Steril* 2011; **96**: 1128-33.

62. ACOG Committee on Practice Bulletins Gynaecology. ACOG Practice BulletinNo. 108: Polycystic ovary syndrome. *Obstet Gynecol* 2009; **114**: 936-49.

63. Bourg CA, Phillips BB. Rosiglitazone, myocardial ischemic risk, and recent regulatory actions. *Ann Pharmacother* 2012; **46**: 282-9

64. Baillargeon JP, Iuorno MJ, Jakubowicz DJ, Apridonidze T, He N, Nestler JE. Metformin therapy increases insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2004; **89**: 242–49.

65. Goel M., Azev VN, d'Alarcao M. The biological activity of structurally defined inositol glycans. *Future Med Chem* 2009; **1**(1): 95-118.

66. Ortmeyer HK. Dietary myoinositol results in lower urine glucoseand in lower postprandial plasma glucose in obese insulin resistantrhesus monkeys. *Obes Res* 1996; **4**: 569–75.

67. Baillargeon JP, Nestler JE, Ostlund RE, Apridonidze T, Diamanti-Kandarakis E. Greek hyperinsulinemic women,with or without polycystic ovary syndrome, display altered inositols metabolism. *Hum Reprod* 2008; **23**: 1439–46.

68. Baillargeon JP, Iuorno MJ, Apridonidze T, Nestler JE. Uncoupling between insulin and release of a D-Chiro-Inositol-Containing Inositol phosphoglycan mediator of insulin action in obese women with Polycystic Ovary Syndrome. *Metab Syndr Relat Disord* 2010; **8**: 127-36.

69. Iuorno MJ, Jakubowicz DJ, Baillargeon JP, Dillon P, Gunn RD, Allan G, Nestler JE. Effects of D-chiro-inositol in lean women with the polycystic ovary syndrome. *Endocr Pract* 2002; **8**: 417–23.

70. 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.

71. 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

72. Cheang KI, Baillargeon JP, Essah PA, Ostlund RE Jr, Apridonize T, Islam L, Nestler JE. Insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator correlates with insulin sensitivity in women with polycystic ovary syndrome. *Metabolism* 2008; **57**: 1390–7

73. Isabella R, Raffone E. Does ovary need D-chiro-inositol? *J Ovarian Res* 2012; **5**: 14.

74. Palomba S, Falbo A, Di Cello A, Cappiello F, Tolino A, Zullo F. Does metformin affect the ovarian response to gonadotropins for in vitro fertilization treatment in patients with polycystic ovary syndrome and reduced ovarian reserve? A randomized controlled trial. *Fertil Steril* 2011; **96**: 1128-33.

75. Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F. Insulin stimulates testosterone biosynthesis by humanthecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. *J Clin Endocrinol Metab* 1998; **83**: 2001–5.

76. Rubin LJ, Hale CC. Characterization of a Mg-dependent, Na-inositol co-transport process in cardiac sarcolemmal vesicles. *J Mol Cell Cardiol* 1993, **25**(6): 721-31.

77. Unfer V, Carlomagno G, Papaleo E, Vailati S, Candiani M, Baillargeon JP. Hyperinsulinemia alters myoinositol to D-chiroinositol ratio in the follicular fluid of patients with PCOS. *Reprod Sci* 2014; **21**: 854-8.

78. Asplin I, Galasko G, Larner J. chiro-Inositol deficiency and insulin resistance: a comparison of the chiro-inositol- and the myo-inositol-containing insulin mediators isolated from urine, hemodialysate, and muscle of control and type II diabetic subjects. *Proc Natl Acad Sci USA* 90 1993; **90**(13):5924-8.

79. Rice S, Christoforidis N, Gadd C, Nikolaou D, Seyani L, Donaldson A, Margara R, Hardy K, Franks S. Impaired insulin-dependent glucose metabolism in granulosa-lutein cells from anovulatory women with polycystic ovaries. *Hum Reprod* 2005; **20**: 373–81.

80. 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. *Endocr J* 2013; **61**: 111–7.

81. Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. *Fertil Steril* 2011; **95**: 2515–6.

82. Vitagliano A, Quaranta M Noventa M, Gizzo S. ''Empiric'' inositol supplementation in normal-weight non insulin resistant women with polycystic ovarian disease: from the absence of benefit to the potential adverse effects. *Arch Gynecol Obstet* 2015; **291**(5):955-7.

83. Croze ML, Vella RE, Pillon NJ, Soula HA, Hadji L, Guichardant M, Soulage CO. Chronic treatment with myo-inositol reduces white adipose tissue accretion and improves insulin sensitivity in female mice. *J Nutr Biochem* 2013; **24**(2): 457-66.

84. Dang NT, Mukai R, Yoshida K, Ashida H. D-pinitol and myo-inositol stimulate translocation of glucose transporter 4 in skeletal muscle of C57BL/6 mice. *Biosci Biotechnol Biochem* 2010; **74**(5): 1062-7.

85. 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.

86. Kamenov Z, Kolarov G, Gateva A, Carlomagno G, Genazzani AD. Ovulation induction with myo-inositol alone and in combination with clomiphene citrate in polycystic ovarian syndrome patients with insulin resistance. Gynecol *Endocrinol* 2015, **31**(2): 131-5.

87. 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.

88. Genazzani AD, Lanzoni C, Ricchieri F, Jasonni VM. Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. *Gynecol Endocrinol* 2008; **24**: 139–44.

89. Morgante G, Orvieto R, Di Sabatino A, Musacchio MC, De Leo V. The role of inositol supplementation in patients with polycystic ovary syndrome, with insulin resistance, undergoing the low-dose gonadotropin ovulation induction regimen. *Fertil Steril* 2011; **95**: 2642–4.

90. Minozzi M, D'Andrea G, Unfer V. Treatment of hirsutism with myo-inositol: a prospective clinical study. *Reprod Biomed Online* 2008; **17**: 579–82.

91. Facchinetti F, Dante G, Neri I. The Ratio of MI to DCI and Its Impact in the Treatment of Polycystic Ovary Syndrome: Experimental and Literature Evidences. *Frontiers in Gynaecological Endocrinology* 2015; ISGE Series: 103-9.

* 92. Dinicola S, Chiu TT, Unfer V, Carlomagno G, Bizzarri M. The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. *J Clin Pharmacol* 2014; **54**: 1079-92.

93. 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.

94. Janovick JA, Conn PM. Gonadotropin-releasing hormone (GnRH)-receptor coupling to inositol phosphate and prolactin production in GH3 cells stably transfected with rat GnRH receptor complementary deoxyribonucleic acid. *Endocrinology* 1994; **135**: 2214-9.

95. Nestler JE. Insulin and insulin-like growth factor-I stimulate the 3 beta-hydroxysteroid dehydrogenase activity of human placental cytotrophoblasts. *Endocrinology* 1989; **125**: 2127-33.

96. Nestler JE. Modulation of aromatase and P450 cholesterol side-chain cleavage enzyme activities of human placental cytotrophoblasts by insulin and insulin-like growth factor I. *Endocrinology* 1987; **121**: 1845-52.

97. Noventa M, Quaranta M, Vitagliano A, Cinthya V, Valentini R, Campagnaro T, Marci R, Di Paola R, Alviggi C, Gangemi M, Saccardi C, Nardelli GB, Gizzo S. May Underdiagnosed Nutrition Imbalances Be Responsible for a Portion of So-Called Unexplained Infertility? From Diagnosis to Potential Treatment Options. *Reprod Sci* 2015 Dec 20; pii: 1933719115620496.

98. Bizzarri M, Cucina A, Harrath AH, Unfer V, Bevilacqua A. Does Myo-Inositol effect on PCOS follicles involve cytoskeleton regulation? *Med Hypotheses* 2016 (in press).

* 99. Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. Hum *Reprod Update* 2004; **10**: 107-17.

* 100. Nestler JE, Unfer V. Reflections on inositol(s) for PCOS therapy: steps toward success. *Gynecol Endocrinol* 2015; **31**: 501-5.

101. 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.

102. Minozzi M, Nordio M, Pajalich R. The Combined therapy myo-inositol plus D-Chiro-inositol, in a physiological ratio, reduces the cardiovascular risk by improving the lipid profile in PCOS patients. *Eur Rev Med Pharmacol Sci* 2013; **17**: 537-40.

* 103. Bevilacqua A, Carlomagno G, Gerli S, Montanino Oliva M, Devroey P, Lanzone A, Soulange C, Facchinetti F, Carlo Di Renzo G, Bizzarri M, Hod M, Cavalli P, D'Anna R, Benvenga S, Chiu TT, Kamenov ZA. Results from the International Consensus Conference on myo-inositol and D-chiro-inositol in Obstetrics and Gynecology--assisted reproduction technology. *Gynecol Endocrinol* 2015; **31**(6): 441-6.

* 104. Croze ML, Soulage CO. Potential role and therapeutic interests of myo-inositol in metabolic diseases. *Biochimie* 2013, **95**(10): 1811-27.

Fig. 1 - Inositol-based molecular pathways of ovarian cells.

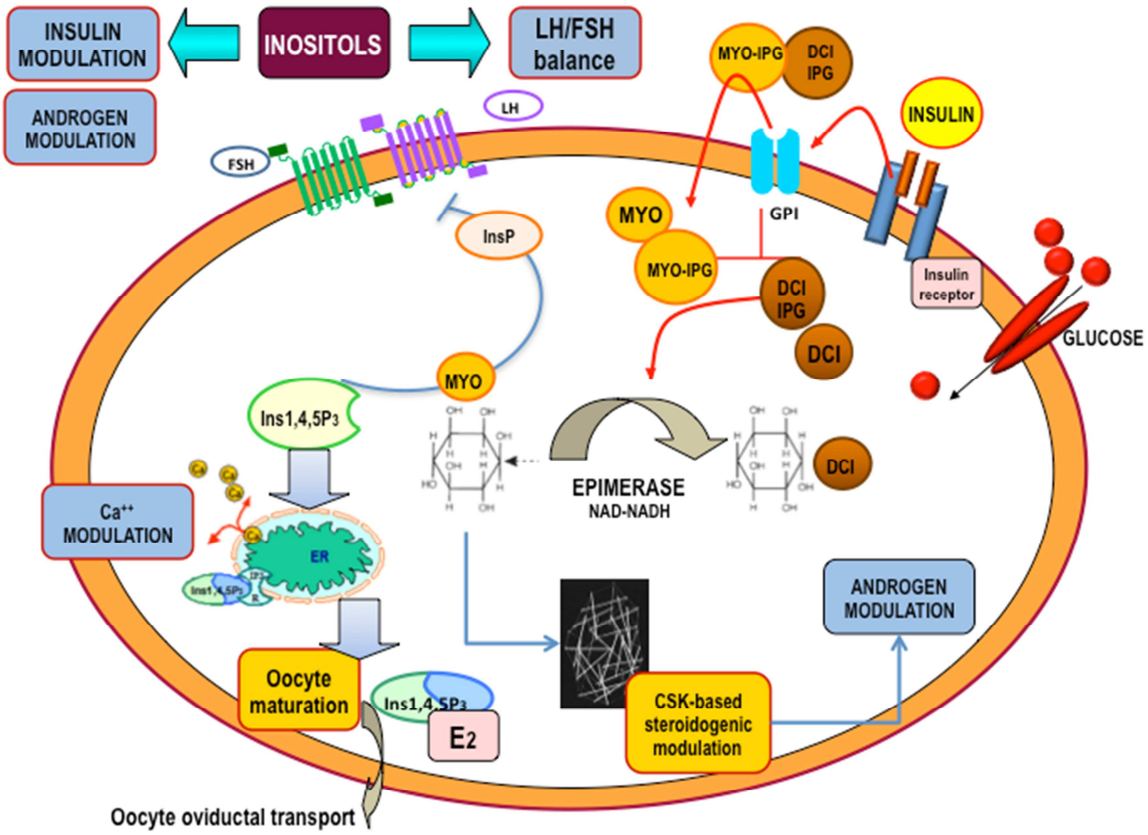
Inositols are involved in structural and biochemical cellular pathways in the ovary. Both D-chiroIns (DCI) and myoIns (MYO) act, as downstream insulin-receptor effectors in promoting glucose transport and insulin-signaling through inositol-based phosphoglycans (DCI-IPG and MYO-IPG). Inositol-phosphoglycan have been found in the cytosol as well as in the outer cell membrane layer, anchored to a glycosyl-phosphatidyl-Inositol (GPI) complex. In addition, insulin fosters the NAD-NADH-dependent epimerase activity, thus enabling MYO to DCI conversion.

MyoIns-phosphate derivative 1,4,5-Inositol-trisphosphate (1,4,5-P3) modulates calcium (Ca^{++}) release form the Endoplasmic Reticulum (ER), by interacting with a specific 1,4,5-Inositol-trisphosphate-receptor (IP3R).

Estrogens (E2) require 1,4,5-P3 for oocyte maturation and oocyte trasport along the oviductal system during fertilization.

MyoIns also modulates LH and FSH signaling (mainly through different inositol-phosphates, InsP), whereas the effect of D-chiroIns CI in signal transduction elicited by these hormones is still controversial.

MyoIns has a role in cytoskeleton (CSK) remodeling that occurs in response to various metabolic stresses and during oocyte maturation. In turn, CSK participates in regulating ovarian steroidogenesis in granulosa and theca cells.



Highlights

- a safe and useful Ins treatment for non insulin-resistent PCOS women is proposed
- the treatment consists of the association of myo-inositol + D-chiro-inositol at a ratio corresponding to their physiological plasma ratio of 40:1
- the combined myo-inositol + D-chiro-inositol administration is safe and provides better results than conventional treatment of PCOS women at both the systemic and ovarian level