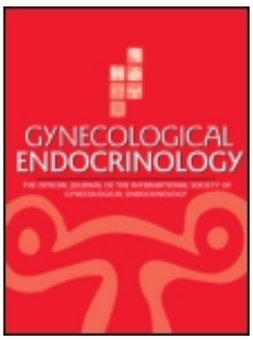

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ORIGINAL ARTICLE

Reflections on inositol(s) for PCOS therapy: steps toward success

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Abstract

In polycystic ovary syndrome (PCOS) pathogenesis, both the insulin resistance and the related compensatory hyperinsulinemia are involved. Despite their similarities, Myo-inositol (MI) and D-chiro-inositol (DCI) play different roles in PCOS etiology and therapy. Indeed, in tissue such as the liver both molecules are involved in the insulin signaling, i.e. MI promotes glucose uptake and DCI glycogen synthesis. In reproductive tissue such as the ovary, MI regulates glucose uptake and follicle stimulating hormone (FSH) signaling, whereas DCI is devoted to the insulin-mediated androgen production. The new hypothesis on "DCI paradox" in the ovary has provided the key for a better understanding. Unlike other tissues, ovary is not insulin resistant, indeed because the epimerase enzyme, which converts MI to DCI, is insulin dependent, the "DCI paradox" hypothesis suggests that in the ovary of PCOS women, an increased epimerase activity leads to a DCI overproduction and MI depletion. This imbalance could be the cause of the poor oocyte quality and the impairment in the FSH signaling. Owing to this situation, the focal point is the administration of both MI and DCI in a proper ratio for treating PCOS. This topic, with several other "hot" issues, was the driving thread in the discussion between the two scientists.

Keywords

D-chiro-inositol, DCI paradox, infertility, insulin resistance, myo-inositol, ovary, polycystic ovary syndrome

History

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Introduction

Scientific progress is mainly based on the exchange of ideas, discussions, debates, and even heated arguments. Without continuous and open communication among scientists, the level of biology, medicine, pharmacology and so on would be much less advanced. Hypotheses have to be tested, compared, analyzed by the scientific community, and eventually accepted or refused.

A compelling example of this process is the evolution of research directed at clarifying the pathophysiologic mechanisms underlying the polycystic ovary syndrome (PCOS) and identifying the optimal therapy for the disorder. This includes the recent exchange of opinions between two scientists, the American endocrinologist John Nestler and the Italian gynecologist Vittorio Unfer, both long active and involved in the study and therapy of PCOS. In particular, their recent discussions, conducted by e-mail over a several months, concerned the role of two stereoisomers of inositol, myo-inositol (MI) and D-chiro-inositol (DCI) in PCOS. It was an engaging scientific exchange that merits widespread dissemination.

Polycystic ovary syndrome (PCOS)

PCOS was first described by Drs. Stein and Leventhal in 1935 [1]. The syndrome affects upto 10% of women of reproductive age, and is the most common cause of infertility in industrialized countries. PCOS is associated with metabolic and hormonal impairments, ovarian dysfunction, and menstrual irregularity. Its current definition requires the presence of two of the three following criteria: (1) chronic oligo-ovulation or anovulation, (2) hyperandrogenism (either clinically established or confirmed by laboratory testing); and (3) the presence of $\geq$12 follicles measuring 2–9 mm in diameter in each ovary and/or increased ovarian volume ($\geq$10 ml), detected by ultrasound examination [2].

There are several hypotheses about the pathogenesis of PCOS invoking various etiological factors, but the issue remains unclear and is the subject of vigorous debate. In the past decade, substantial *in vitro* and *in vivo* evidence has supported the pivotal role of insulin resistance and/or compensatory hyperinsulinemia in the pathogenesis of PCOS (which is present in approximately 80% of obese women with PCOS and 30–40% of lean women with PCOS) [3,4]. The demonstrated efficacy of insulin-sensitizing drugs, such as metformin, troglitazone and pioglitazone, in improving ovulatory function and reducing androgen excess in PCOS provided additional proof to support insulin resistance's pathogenic role in PCOS. However, side effects such as nausea and diarrhea (in the case of metformin) and increased body weight (in the case of pioglitazone) may reduce patients' compliance and limit the use of these drugs [4–6].

Inositol(s) in trials on PCOS

Research on other effective therapeutic options has included the utility of inositol; inositol being a six-carbon ring compound with a hydroxyl group attached to each carbon of the ring. There are nine possible stereoisomeric forms of inositol, related to the epimerization of the six hydroxy groups [7–10]. The rationale underlying the use of inositols as a therapeutic application in PCOS derives from their activities as insulin mimetic (or ''insulin sensitizing'') agents and their salutary effects on metabolism.

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We highlight that two specific stereoisomers of inositol, MI and DCI, both function as insulin second messengers and mediate different actions of insulin. MI is converted to an inositolphosphoglycan (IPG) insulin second messenger (MI-IPG) involved in cellular glucose uptake, whereas DCI is converted to an IPG insulin second messenger (DCI-IPG) involved in glycogen synthesis [11]. On the other hand, at ovarian level it has been shown that MI based second messenger is involved in both glucose uptake and FSH signaling whereas DCI-based second messenger is devoted to the insulin-mediated androgen production. Previous studies, performed by John Nestler and his team [12], provided evidence that the impairment in insulin signaling in PCOS could be the result of a defect in the IPG insulin second messenger pathway, consistent with the insulinomimetic role of IPGs in activating enzymes controlling glucose metabolism. In women with PCOS, a deficiency of IPGs in tissues, or altered metabolism of inositols to IPG mediators, could play a role in inducing insulin resistance [13]. In the first controlled clinical trial of inositols in PCOS, 1200 mg of DCI or placebo, given orally once a day for 6–8 weeks to 44 obese PCOS women, improved insulin sensitivity and decreased circulating free testosterone levels, whereas there was no effect of placebo. DCI administration also resulted in ovulation in 19 of 22 women (86%), whereas only 6 of 22 women (27%) ovulated in the placebo group [13]. After publication of this intriguing report, in 1998, Insmed Pharmaceuticals obtained a U.S. patent claiming the effectiveness of DCI in the treatment of PCOS, and, in 2002, a follow-up study was performed by the same group in lean women with PCOS [14]. Again and consonant with the earlier study [13], administration of DCI was associated with improved insulin sensitivity, a reduction in circulating free testosterone, and increased frequency of ovulation [14].

Insmed Pharmaceuticals subsequently embarked on a large multi-center placebo-controlled trial of DCI in women with PCOS, using a dose of DCI twice as high as ever used previously namely 2400 mg. While the results were never published, in September 2002 Insmed Pharmaceuticals announced that:

> ''In recently completed clinical trials in patients with PCOS, INS-1 [DCI] was safe and well tolerated but did not achieve statistical significance on its primary efficacy measures. Although an overall increase in ovulation rates was not achieved, an increased number of pregnancies occurred in the INS-1 treated patients. The company is currently evaluating the clinical relevance of this observation and whether it warrants further investigation.''

These results were surprising, since the higher dose of DCI failed to replicate the findings of the two previous studies [12], at least in terms of improving ovulatory frequency. As will be discussed subsequently, the lack of efficacy in the latter trial may have been related to the high dose of DCI administered.

The different role of myo-inositol (MI) and D-chiro-inositol (DCI)

In this regard, previous studies had highlighted the pivotal role of administration of MI for enhancing the success of *in vitro* human fertilization (IVF) in PCOS [15]. It was also reported that follicular fluid (FF) volume and its content of MI were significantly higher in follicles with matured and fertilized oocytes compared with follicles with immature and unfertilized oocytes. Furthermore, the levels of MI in FF were positively correlated with embryo quality [16].

In 2007, an uncontrolled clinical trial of MI administration to 25 PCOS patients, conducted by an Italian research group headed by Vittorio Unfer, produced new data on the possible physiologic function and therapeutic usefulness of MI. They reported an increase in frequency of spontaneous menstrual cycles and eventual pregnancies in the women with PCOS who received MI in combination with folic acid, and suggested on this basis that MI may have utility in the treatment of infertility in PCOS [17]. Several follow-up studies supported these findings and the idea that MI exerts beneficial effects on ovulation and oocyte quality [18–21]. Of note, MI administration to women with PCOS undergoing IVF was associated with a reduction in the quantity of recombinant FSH (rFSH) administered and the number of days of stimulation [22,23]. These evidences demonstrate that MI improves FSH sensitivity lending further support to the idea that MI administration beneficially affects ovarian function and oocyte development.

Unfer and coworkers conducted a comparative study of the effects of administration of MI versus DCI on oocyte quality in PCOS patients. They reported that the number of mature oocytes was significantly higher, with a parallel diminution in the number of immature oocytes, in the MI group compared to the DCI group, even though the total number of oocytes retrieved did not differ between the two treatment groups [23]. A potential explanation for this phenomenon was the tissue-specific nature of insulin resistance in women with PCOS. Indeed, although muscle and liver are insulin-resistant in women with PCOS, the ovaries retain normal insulin sensitivity [24–26].

The ''DCI paradox'' in the ovary

Unfer, Carlomagno and Roseff published a letter positing the tissue-specificity of insulin resistance in PCOS as the underpinning of the ''DCI paradox'' in the ovary [27]. The epimerase enzyme converts MI to DCI in the ovary, and ovarian epimerase is stimulated by insulin. Unfer et al. suggested that in women with PCOS, hyperinsulinemia likely stimulated epimerase activity in the ovary, resulting in an overproduction of DCI and a concomitant depletion of MI. The authors postulated that the resulting deficiency of MI could be responsible for the poor oocyte quality and the impairment of the FSH signaling. Clearly, DCI supplementation would be ineffective in such women as they already have high levels of this molecule in the ovary. At this juncture, it was incumbent to determine the physiological concentrations of MI and DCI in plasma in normal women to identify the presumably optimal clinical dosage [28]. The ratio of MI to DCI in plasma was found to be approximately 40:1. Based on this information, a treatment was established in the pharmaceutical form of a soft gel capsule containing 550 mg of MI and 13.8 mg of DCI, patented by Lo.Li. Pharma [28]. It was anticipated that the synergetic activity of the two stereoisomers would result in a (1) DCI-mediated enhancement of insulin sensitivity in liver and muscle resulting in a decrease in circulating insulin, and (2) repletion of MI in the ovary, resulting in restored FSH sensitivity and improved oocyte quality. A study by Nordio et al. [29] supported this hypothesis and the subsequent recent Inositol Consensus Conference on the use of MI and DCI in obstetrics, gynecology and fertility [30], held in Florence, formally affirmed the framework suggested by Unfer, adding a new milestone to the promising story of inositol.

A fruitful scientific debate

As noted at the start of this article, a noteworthy step in our understanding of inositols in PCOS has been the recent correspondence between the Italian scientist, Vittorio Unfer, and the American scientist, John Nestler. After the publication of the review by Unfer and Porcaro [31], Nestler wrote to congratulate them on the review. Later, he received from Unfer a copy of the

Inositol Consensus Conference draft, and was invited to share his opinion. Nestler expressed his appreciation and responded that he found the ''DCI paradox'' hypothesis both ''intriguing and attractive.'' Afterwards the exchange between these two scientists went straight to the heart of the matter.

Several points were raised by the American endocrinologist. The first one concerned the possibility of further testing the idea that women with PCOS maintain normal ovarian sensitivity to insulin and, hence, that hyperinsulinemia stimulates ovarian epimerase activity in PCOS women, by sampling FF before and after an intervention to lower circulating insulin (e.g. using diazoxide or acarbose). If this study was not feasible, Nestler suggested sampling FF from two groups of PCOS women, one group treated with an insulin-lowering drug and the other one receiving a placebo, to verify if there is a difference between the two groups in the ratio of MI to DCI in FF. Unfer agreed that sampling FF before and after an intervention to reduce circulating insulin could be informative, but noted that the study would be difficult to conduct, since it would require obtaining FF specimens twice from the same PCOS women during two separate spontaneous cycle. Furthermore, MI and DCI levels in FF likely vary during the different phases of the menstrual cycle. Indeed, ovarian MI synthesis is hormonally regulated, likely because optimal FSH signaling requires MI. Therefore, even the second proposed study would be difficult to perform.

A second point regarded the implications of the ''DCI paradox'' hypothesis. According to the hypothesis, classic insulin target tissues in PCOS women are DCI deficient (because of resistance to insulin's physiologic stimulation of epimerase), whereas the opposite (DCI overloaded and MI deficient) is the case in the ovary since it does not become insulin-resistant and hyperinsulinemia excessively stimulates epimerase. Nestler noted that it has been reported that the ratio of the DCI-containing IPG second messenger (DCI-IPG) released per unit insulin released increases with metformin treatment in PCOS women [31]. Based on this finding, Nestler inquired whether there is any evidence that metformin directly stimulates epimerase activity *in vitro* or *in vivo*, or if there might be an alternative explanation for this phenomenon. Unfer was not aware of any studies addressing this query.

Finally, Nestler then inquired if Unfer would speculate as to why the initial studies of Nestler and colleagues of DCI administration to PCOS women were positive, whereas the follow-up Insmed Pharmaceuticals study that doubled the dose of DCI was not? Nestler wondered if genetic or dietary factors could explain the disparate results, since the initial studies were conducted in Venezuelan women and the subsequent study was conducted in the U.S. Unfer replied that the study employing the lower dose of DCI may have restored normal insulin sensitivity in classic insulin target tissues, such as liver and muscle, which would then reduce circulating insulin levels. The noted improvement in ovulatory frequency in these studies could then be attributed to the overall improvement in insulin sensitivity and reduction in circulating insulin. In contrast, in the study using a higher dose of DCI, it is possible that the ratio of MYO to DCI in FF was so altered as to impair the ovulatory process. Hence, there may be biphasic response to DCI in PCOS.

Unfer further noted that optimal treatment of PCOS may require administration of a combination of MI and DCI. Indeed, according to the ''DCI paradox'' hypothesis, we have to deal with two different targets, the first ones are the classic insulin target tissues (liver, muscle and fat) that need to restore insulin sensitivity, while the second one, the ovary, needs to re-establish glucose uptake, FSH signaling and to reduce testosterone production. Both these targets are accomplished by administering MI and DCI in the proper ratio. Indeed, in liver, muscle and fat MI and DCI cooperate to re-establish insulin sensitivity, whereas the administration of MI + DCI, in their physiological ratio, restores glucose uptake and FSH signaling (thanks to MI) and reduces the androgen production. In Unfer's opinion, administering only DCI could induce an imbalance in the ratio of MI to DCI at the ovarian level, hindering effective FSH signaling. He wondered if Nestler had data on the use of DCI in non-insulin resistant PCOS women, because Unfer hypothesized that in such women DCI should not exert beneficial effects while MI would. Unfortunately, no such data were available; Nestler had only the published data of metformin's salutary effects in lean women with PCOS who appeared insulin-sensitive [32]. The final comment of the American scientist was on the need to better understand the possible connection between the ''DCI paradox'' and the previously cited data for metformin.

DCI and ''FSH resistance''

Relevant to this discussion, Unfer noted that, in an as yet unpublished preliminary study conducted by his team, the majority of PCOS women treated with 500 mg DCI twice a day re-experienced amenorrhea after 6 months of treatment. Furthermore, their menstrual cycle was restored in 2 months once the treatment had been discontinued. Unfer's hypothesis is that administration of a high dosage of DCI could dramatically and adversely alter the plasma ratio of MI to DCI. The increase in plasma DCI would likely saturate the cellular transporter for MI, increasing intra-ovarian concentrations of DCI. Such enhancement would result in a reduction of the intra-ovarian concentration of MI that, in turn, would induce an ''FSH resistance.'' In response, Nestler noted that in his original DCI paper, published in the *New England Journal of Medicine* [33], there was a marked rise in ovulation in the women treated with ''low-dose DCI'' compared with the placebo group, with about 85% of the DCI women ovulating, but that there are no data on what happened after the cessation of DCI treatment since the women were not followed-up afterwards. The American scientist deemed Unfer's hypothesis of ''high-dose DCI'' saturating the MI transporter as provocative, and noted that the scientific question remains whether the MI transporter transports DCI as well.

Unfer then moved the subject of their discussion to the possible association between PCOS and depression and/or eating disorders, because some scientific papers have addressed this topic. Nestler stressed that such studies are limited in number. In his clinical practice, the impression was that any depression originates primarily from the marked obesity and poor self-image of the patient, rather than from the PCOS state itself. Indeed, only on a rare occasion has he referred a PCOS patient to a psychiatrist. No psychological or psychiatric assessments were performed in his DCI or other PCOS-related research studies.

Other issues raised concerned which molecule, DCI or MI, would be most effective in preventing the development of gestational diabetes mellitus (GDM) in PCOS. In Nestler's opinion, since the focus in GDM would be on ameliorating insulin sensitivity and enhancing glucose disposal, and not on reproductive consequences, restoring DCI sufficiency in classic insulin target tissues might be more important. For Nestler, one corollary to the ''DCI paradox'' hypothesis is that we can decide to administer MI or DCI depending on which tissue needs to be ''remedied'' (i.e. classic insulin target tissue versus ovary). In turn, Unfer replied that, if the focus is solely on metabolic aspects and not on the ovary, DCI could be an effective therapeutic approach in the prevention or treatment of GDM in PCOS

patients. However, Unfer highlighted that the combination of MI plus DCI, administered at a physiological ratio, may be ultimately the better therapeutic option since it may also address the energetic needs of the fetus. The American endocrinologist agreed that the appropriate (physiological) ratio of MI to DCI should be administered to PCOS patients, in this way reaching the effects at different levels. As a whole, Nestler observed that, in fact, their respective points of view were not divergent; indeed, he very much appreciated the scientific underpinning and therapeutic implications of Unfer's ''DCI paradox'' hypothesis.

Finally, Nestler updated Unfer that his group had recently completed analyzing data from a study where they treated PCOS women with pioglitazone or placebo, to determine whether pioglitazone would influence the metabolism of DCI by decreasing urinary clearance of DCI. No such effect was noted, but they did observe that pioglitazione treatment significantly increased the ratio of DCI-IPG to insulin released during an OGTT ($AUC_{DCI\text{-}IPG}/AUC_{insulin}$), which means that more DCI-IPG was released per unit insulin [34]. This result matches those previously observed with metformin [35].

Unfortunately, nowadays there are no technical means to perform MI-IPG dosage.

Toward some shared conclusions

In Nestler's opinion, these observations with two different classes of insulin-sensitizing drugs (metformin and pioglitazone), can be explained in several ways. These drugs could (1) improve the ''coupling'' of DCI-IPG release to insulin release; (2) promote the formation of DCI-IPG second messenger from existing intraovarian DCI substrate; (3) stimulate ovarian epimerase activity directly, increasing DCI substrate; or (4) enhance the sensitivity of ovarian epimerase to stimulation by insulin. A final possibility could be that these results are an epiphenomenon related to some other factor(s), not so far considered.

Unfer speculated that metformin might improve insulin sensitivity through a direct stimulatory action on the epimerase enzyme. Indeed, Palomba et al. [36] suggested that metformin may worsen the ovarian response to FSH (which would be the case if it increased intraovarian DCI and depleted MI), while at the hepatic level metformin seemed to increase glycogen synthesis. Therefore, Unfer noted, the beneficial effects of metformin on insulin resistance may relate to increased intra-tissue production of DCI (and, consequently, DCI-IPG) that induces glycogen synthesis in the liver and muscle, while the initial beneficial effect at the ovarian level might be more indirectly linked to the consequent reduction in circulating insulin. Nestler responded that metformin's effect specifically on ovarian epimerase is unknown, and that theoretically it could be the opposite in the ovary from the effect it might have in classical insulin target tissues. For the American scientist, this is a ripe area for investigation.

Unfer speculated that it remains to be elucidated whether prolonged or high metformin dosage might have the same negative effects as the ones observed with DCI. However, Nestler noted that, in his clinical experience, metformin's beneficial effects on ovulation in PCOS women who are ''responders,'' when administered at a dose of 2000 mg daily, has been sustained even after 10 or more years of treatment.

This brought the exchange of ideas between the two scientists to the conclusion that their correspondence was not about the sharing of ''divergent views,'' since the exchange resulted at its core in substantial agreement on the ''DCI paradox'' hypothesis. Their conversation proved to be a thought-provoking reflection on the topic of inositols in PCOS, and identified several questions that merit scientific inquiry. This is yet another example of how a healthy exchange of ideas can advance science.

Declaration of interest

Vittorio Unfer is an employee at LO.LI. Pharma, Rome. John Nestler reports no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

1. Stein I, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 1935;29:181–91.
2. Koivunen R, Laatikainen T, Tomás C, et al. The prevalence of polycystic ovaries in healthy women. Acta Obstet Gynecol Scand 1999;78:137–41.
3. Nestler JE. Role of hyperinsulinemia in the pathogenesis of the polycystic ovary syndrome, and its clinical implications. Semin Reprod Endocrinol 1997;15:111–22.
4. Baillargeon JP, Iuorno MJ, Nestler JE. Insulin sensitizers for polycystic ovary syndrome. Clin Obstet Gynecol 2003;46:325–40.
5. Genazzani AD, Lanzoni C, Ricchieri F, et al. Metformin administration is more effective when non-obese patients with polycystic ovary syndrome show both hyperandrogenism and hyperinsulinemia. Gynecol Endocrinol 2007;23:146–52.
6. Pasquali R, Gambineri A. Insulin-sensitizing agents in polycystic ovary syndrome. Eur J Endocrinol 2006;154:763–75.
7. Wakelam M, ed. The cell biology of inositol lipids and phosphates. Proceedings of the 2006 Biochemical Society Annual Symposium; 2006 Mar 29–30; Birmingham, United Kingdom; Biochemical Society Symposium 2007;74:1–271.
8. Agranoff BW. Turtles all the way: reflections on myo-inositol. J Biol Chem 2009;284:21121–6.
9. Johnson LF, Tate ME. The structure of myo-inositol pentaphosphates. Ann N Y Acad Sci 1969;165:526–32.
10. Raboy V. Myo-inositol-1,2,3,4,5,6-hexakisphosphate. Phytochemistry 2003;64:1033–43.
11. Larner J, Huang LC, Tang G, et al. Insulin mediators: structure and formation. Cold Spring Harb Symp Quant Biol 1988;53:965–71.
12. Cheang KI, Baillargeon JP, Essah PA, et al. 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.
13. Baillargeon JP, Diamanti-Kandarakis E, Ostlund RE Jr, et al. Altered D-chiro-inositol urinary clearance in women with polycystic ovary syndrome. Diabetes Care 2006;29:300–5.
14. Iuorno MJ, Jakubowicz DJ, Baillargeon JP, et al. Effects of D-chiro-inositol in lean women with the polycystic ovary syndrome. Endocr Pract 2002;8:417–23.
15. 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.
16. 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.
17. 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.
18. 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.
19. 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.
20. Zacchè 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.
21. 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.
22. 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.
23. 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.
24. Harwood K, Vuguin P, DiMartino-Nardi J. Current approaches to the diagnosis and treatment of polycystic ovarian syndrome in youth. Horm Res 2007;68:209–17.
25. Matalliotakis I, Kourtis A, Koukoura O, Panidis D. Polycystic ovary syndrome: etiology and pathogenesis. Arch Gynecol Obstet 2006; 274:187–97.
26. Rice S, Christoforidis N, Gadd C, et al. Impaired insulin-dependent glucose metabolism in granulosa-lutein cells from anovulatory women with polycystic ovaries. Hum Reprod 2005;20:373–81.
27. Carlomagno G, Unfer V, Roseff S. The D-chiroinositol paradox in the ovary. Fertil Steril 2011;95:2515–16.
28. Carlomagno G, De Grazia S, Unfer V, Manna F. Myo-inositol in a new pharmaceutical form: a step forward to a broader clinical use. Expert Opin Drug Deliv 2012;9:267–71.
29. 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.
30. Bevilacqua A, Carlomagno G, Gerli S, et al. Results from the international consensus conference on myo-inositol and D-chiroinositolin obstetrics and gynecology – assisted reproduction technology. Gynecol Endocrinol 2015. [Epub ahead of print]. doi: 10.3109/09513590.2015.1006616.
31. Unfer V, Porcaro G. Updates on the myo-inositol plus D-chiro-inositol combined therapy in polycystic ovary syndrome. Expert Rev Clin Pharmacol 2014;7:623–31.
32. Baillargeon JP, Iuorno MJ, Jakubowicz DJ, et al. Metformin therapy increases insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004;89:242–9.
33. 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.
34. Gupta A, Jakubowicz DJ, Nestler JE. Pioglitazone therapy increases insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator in women with polycystic ovary syndrome. (Abstract) 97th annual scientific meeting of The Endocrine Society; San Diego, CA, March 5–8, 2015.
35. Nestler JE. Metformin for the treatment of the polycystic ovary syndrome. N Engl J Med 2008;358:47–54.
36. Palomba S, Falbo A, Di Cello A, et al. 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.