



New clinical targets of d-chiro-inositol: rationale and potential applications

Antonio Simone Laganà , Simone Garzon & Vittorio Unfer

To cite this article: Antonio Simone Laganà , Simone Garzon & Vittorio Unfer (2020): New clinical targets of d-chiro-inositol: rationale and potential applications, Expert Opinion on Drug Metabolism & Toxicology, DOI: [10.1080/17425255.2020.1785429](https://doi.org/10.1080/17425255.2020.1785429)

To link to this article: <https://doi.org/10.1080/17425255.2020.1785429>



Accepted author version posted online: 19 Jun 2020.



Submit your article to this journal [↗](#)



Article views: 2



View related articles [↗](#)



View Crossmark data [↗](#)

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Opinion on Drug Metabolism & Toxicology*

DOI: 10.1080/17425255.2020.1785429

Review

New clinical targets of D-chiro-inositol: rationale and potential applications

Antonio Simone Laganà¹, Simone Garzon¹, Vittorio Unfer²

¹ Department of Obstetrics and Gynecology, "Filippo Del Ponte" Hospital, University of Insubria, Piazza Biroldi 1, 21100 Varese, Italy

² Systems Biology Group Lab, "La Sapienza" University, Via A. Scarpa 16, 00163, Rome, Italy

Corresponding author: Antonio Simone Laganà, Department of Obstetrics and Gynecology, "Filippo Del Ponte" Hospital, University of Insubria, Piazza Biroldi 1, 21100 Varese, Italy,
email: antoniosimone.lagana@uninsubria.it

Abstract

Introduction: Inositols have a key role in ovarian physiology and the literature reports a wealth of studies about the major isomer, myo-inositol (MI). However, information about D-chiro-inositol (DCI) is still scarce, despite the ratio MI:DCI is tissue-specific and actively maintained by an insulin-dependent epimerase enzyme.

Areas Covered: This expert opinion provides an overview of the physiological contribution of DCI in regulating steroidogenesis. DCI indeed mediates the intracellular signaling of insulin, which induces the biosynthesis of androgens. Studies on second messengers of insulin also revealed that DCI has a specific role in modulating the activity of aromatase enzyme. Specifically, recent findings demonstrated that DCI influence the enzyme gene expression, thus reducing the conversion of androgens into estrogens.

Expert opinion: available evidence suggests that the effects of DCI administration may be similar to those of aromatase inhibitors, but without causing hypo-estrogenic states. Therefore, DCI treatments should be evaluated for either estrogen-dependent gynecological conditions or low testosterone states in male subjects.

Keywords: D-chiro-inositol, aromatase, estrogen, endometriosis, controlled ovarian hyperstimulation, male hypogonadism.

Article highlights

- D-chiro-inositol (DCI), besides being a second messenger of insulin, regulates female steroidogenesis by enhancing testosterone biosynthesis and downregulating the expression of aromatase.
- As second messenger of insulin, short-term administration of DCI may reduce androgen levels in insulin-resistant women; extended treatments result in androgen increase due to the activity on aromatase and on testosterone biosynthesis.
- Taking into account the modulatory action on aromatase, DCI may find application for estrogen-dependent gynecological diseases characterized by increased expression of the enzyme.
- Tailoring the dosage and the duration of the intervention, DCI treatment may represent an appealing therapeutic approach for increasing the androgen-to-estrogen ratio in men with late-onset hypogonadism.

1. Introduction

In the last years, the role of Inositols in gynecological endocrinology and reproduction has attracted a growing interest [1]. A very recent Expert Opinion reviewed the characteristics of the two major isomers, myo-inositol (MI) and D-chiro-inositol (DCI), discussing possible clinical applications in the field of human reproduction [2]. While MI, the most abundant isomer, has a largely established role in ovarian physiology [3], the current pieces of evidence about DCI's actions are less robust, despite all organs and tissues contain variable amounts of both isomers. A specific epimerase enzyme converts MI into DCI and maintains local MI:DCI ratios, suggesting that DCI is an essential component of cellular Inositol pool and that its concentration is actively regulated. A recent report suggests that MI:DCI ratios may play a key role for several physiological processes [4]. The epimerase has a tissue-specific activity [5], leading to well-defined MI:DCI ratios in different tissues and organs [6]; this ratio is around 40:1 in the peripheral blood [7], about 20:1 in the thecal cells and within the range 70:1 – 100:1 in the follicular fluid of dominant follicles [8, 9]. Defective regulation of the epimerase's activity results in abnormal levels of DCI and altered MI:DCI ratios, leading to impaired steroid biosynthesis [10], associated with several pathological conditions [11]. The present review focuses on the role of DCI in steroidogenesis, in particular from a gynecological perspective, discussing the potential and limitations of DCI treatments to modulate the endogenous production of estrogens and androgens.

2. D-chiro-inositol and steroid biosynthesis

In the phosphoglycan form, DCI is a second messenger of insulin signal [12, 13] and indirectly influences steroidogenesis. Nevertheless, DCI exhibits also an independent activity on androgen biosynthesis, as found by Nestler and co-workers [14]. They demonstrated that a DCI-based phosphatidyl-glycan is able to stimulate the production of testosterone in human thecal cells. The same group also reported that DCI is able to reduce the activity of the enzyme CYP19A1 [15]. Such enzyme, commonly known as aromatase, is a member of the cytochrome P-450 family and catalyzes the oxidative conversion of androgens into estrogens. Consistently, 6-weeks treatment with 2400 mg/day DCI has been found to increase testosterone levels in women with polycystic ovary syndrome (PCOS) [16]. In addition, Sacchi et al. found that DCI affects the gene expression of aromatase, downregulating it in a dose-response manner [17]. These observations lately prompted comparison between the activities of DCI and those of compounds known to inhibit aromatase enzyme [18], although with substantially different mechanisms of action. The aromatase inhibitors (AIs) prevent the biosynthesis of estrogens, leading to a systemic hypoestrogenic state and to potentially negative side effects, such as the loss of bone density [19]. Conversely, DCI modulates the estrogen biosynthesis affecting the androgen-to-estrogen ratio.

3. D-chiro-inositol administration: duration and patients' condition.

The activity of DCI on steroidogenesis, hence, may be two-fold: on the one hand, an indirect effect as a result of insulin signal boosting; on the other hand, an independent direct effect on steroid biosynthesis through downregulation of the aromatase expression and enhancement of testosterone production. Most of the available data regarding treatments with DCI derives from clinical studies on PCOS women, which presented often with associated insulin resistance. In these patients, the administration of low doses of DCI causes systemic reduction of insulin resistance [20]. As a consequence, levels of androgens decrease in the short term [21], accompanied by estrogen decrease if a hyper-estrogenic condition is present. Continued administration of DCI exhibits effects on aromatase and testosterone biosynthesis, with androgen levels that begin to increase again. Elevated dosages of DCI in the same type of patients may act on both

pathways at one time, even for short-term administration, leading to increased androgen levels [16]. Unfortunately, to the best of our knowledge literature data regarding the administration of DCI to non-insulin resistant women are still scarce and do not allow to draw a firm conclusion. However, based on the available information, we would expect a little initial effect on insulin levels, followed by reduced activity of aromatase. On that basis, DCI dose and timing of administration should be carefully evaluated: in particular, dosages of DCI and treatment duration should be tailored to the patients' specific condition, taking into account patients that may potentially suffer from increased androgen levels.

4. Potential clinical applications

Based on the available evidence [18], the use of DCI in gynecology clinical practice is likely to have a rationale for all those conditions that benefit from therapeutic treatments with aromatase inhibitors or insulin sensitizers. Moreover, adjusting the dosage and the duration of treatment, we suggest that the administration of DCI to hypogonadal men, particularly if obese, may have beneficial effects by increasing testosterone and decreasing estradiol.

4.1. Ovulation induction

Accounting for over one-third of infertile couples, the occurrence of anovulatory menstrual cycles represents one of the leading causes for infertility [22]. Long-term anovulation generally induces decreased estrogen levels [23, 24], potentially resulting in reduced bone density and cardiovascular problems. According to the World Health Organization (WHO), group II is the most common type of anovulatory women, including subjects with PCOS [25] and those with altered signaling along the hypothalamic-pituitary-ovarian axis. First-line treatments for this group of patients include oral ovulation-inducing agents, which reduce the biosynthesis of estrogens and consequently decrease the negative hypothalamic feedback on Gonadotropin-Releasing Hormone (GnRH) and follicle-stimulating hormone (FSH) [26]. Such drugs are either selective estrogen receptors modulators, such as clomiphene citrate (CC), or third-generation AI, such as Letrozole or Anastrozole. DCI may induce ovulation in PCOS patients in a similar way to Letrozole [27], and such an effect may be dose-dependent [28]. While low-dosage administration of DCI to PCOS patients exhibits negligible effects, doses as high as 1200 mg/day for 6-8 weeks successfully induced ovulation in up to 44% patients, compared to a control group taking placebo [29]. Such effect seems independent of BMI, as it was observed also with lean women [30]. Despite these pieces of evidence, the underlying mechanism of the ovulation induction after DCI treatment is still controversial. Besides the decreased activity of aromatase, also lowered levels of insulin may reduce estrogens production and release, restoring the physiological functioning of the hypothalamic-pituitary-ovarian axis [31]. Indeed, metformin is able to induce ovulation in PCOS patients as it reduces the insulin-stimulated production of androgens and the insulin-dependent activity of aromatase [32]. Decreased insulin levels also improve the efficacy of ovulation stimulation protocols either with gonadotropins [33] or AIs [34]. In rationalizing the effect of DCI on inducing ovulation, the contribution of both mechanisms should be considered, even though they occur with different timing.

4.2. Ovarian hyperstimulation syndrome (OHSS)

Although quite uncommon, OHSS represents one of the most serious complications of ovulation induction during in vitro fertilization (IVF) protocols. The syndrome is triggered by an exaggerated ovarian response to either exogenous (early-onset) or endogenous (late-onset) human Chorionic Gonadotropin (hCG), which induces the abnormal release of factors that increase vascular permeability [35]. In particular, luteal overexpression of Vascular-Endothelial Growth Factor (VEGF) seems to be directly associated with the onset of OHSS during stimulation protocols [36, 37]. The consequent higher vascular permeability may lead to transudation of protein-rich fluid from the blood vessels mainly into the peritoneum. Pathological features range from mild symptoms, such as abdominal discomfort, to life-threatening complications in the most severe cases. Treatments largely vary, depending on the severity of the clinical manifestations, and generally include cancellation of the stimulation cycle, with a profound economic and psychological burden for the patients [38]. Moreover, pregnancy-related complications are significantly higher in women who had suffered from OHSS, compared to IVF controls [39]. Prevention is therefore paramount during

fertilization programs and requires the assessment of risk factors and close monitoring of risk markers. Clinicians quite generally consider unusually elevated estrogen levels as both indicators for increased risk of OHSS and one possible etiological factor [40]. Strategies to reduce the occurrence of OHSS in subjects at risk include postponing (*coasting*) the hCG injection until the estradiol levels fall below a safe threshold [41]. Estrogen reduction with short-term Letrozole treatment (5 days) before hCG administration proved to significantly reduce the incidence of OHSS in high-risk women undergoing assisted reproduction [42]. Interestingly, analogous results were obtained with insulin sensitizers. Indeed, longer-term treatment (>30 days) with metformin during ovarian stimulation also significantly reduced the risk of OHSS [43]. In this context, the potential use of DCI as a primary strategy, or more likely as an adjuvant treatment to the current management, may help to reduce the hypoestrogenism and the risk of OHSS.

4.3. Uterine leiomyomas (fibroids)

These are common benign tumors of the uterus and affect 20-30% of women in the reproductive age. Studies on leiomyoma cells found over-expression of the enzyme aromatase, compared with normal cells of the myometrium [44], with consequent high estrogen levels and increased expression of progesterone receptors [45]. Even though surgery represents the main curative treatment for large and symptomatic leiomyomas [46, 47], the management of symptoms prevents stress and surgery-related complications. Reducing estrogen levels has been found particularly useful, as a hypoestrogenic environment proved to inhibit the proliferation of uterine myoma cells [48] and to reduce the size of uterine leiomyomas in women after the menopausal transition [49]. Treatment with aromatase inhibitors, such as Letrozole, was more effective than hormonal therapy in reducing fibroid volume and decreasing the duration and the volume of fluid absorbed during surgery [50]. On that basis, the use of DCI may reduce the pro-estrogenic microenvironment at level of the uterine myoma cells, and alleviate symptoms and signs by providing a potential effective adjuvant strategy to reduce fibroids' volume.

4.4. Endometriosis

The abnormal growth of endometrial-like tissue, glands, and stroma outside the uterine cavity is a frequent gynecological disorder that causes pelvic pain and may lead to infertility [51]. Endometriosis occurs primarily in women of reproductive age and tends to disappear after menopause when endogenous estrogen production decreases. However, recurrent endometriosis may be also detected in post-menopausal women. Researchers demonstrated that both ectopic and eutopic endometrial tissues in women with endometriosis express high levels of aromatase [52, 53, 54], causing the progression of this estrogen-dependent disease and the occurrence of a pro-inflammatory microenvironment [55]. Thus, the endometrium becomes a major intracrine source of estrogen, stimulating the progress of the disease [56]. Indeed, reducing systemic levels of estrogens halts the growth of endometriotic tissue, relieving the pain symptoms [57]. Recent findings suggest that drugs that inhibit the aromatase activity, such as Letrozole and Anastrozole, may have potential benefits in the treatment of endometriosis [58, 59, 60]. Indeed, they proved to significantly reduce both pelvic pain, lesion size, and endometrioma volume. However, AI treatments are more expensive and less tolerable than conventional therapies [61]. Similarly, insulin sensitizers seem to have beneficial effects on endometriosis status, comparable to AIs in the animal model: indeed, both *in-vitro* and *in-vivo* studies on rat endometriosis model demonstrated that treatment with metformin reduces the inflammatory response and the activation of aromatase, and stimulates the regression of endometriotic lesions [62, 63]. In this scenario, by direct and indirect inhibition of aromatases, DCI treatment may play a beneficial role to reduce the pro-estrogenic local microenvironment typical of endometriosis and, most important, could be used in association with other drugs in order to reduce their doses and consequently the known potential side-effects.

4.5. Breast and endometrial tumors.

Both types of tumors have several risk factors in common. The majority of breast cancers and type-1 endometrial cancer express estrogen receptors and the risk of both is higher in women with elevated endogenous estrogen levels [64, 65]. Moreover, both *in vitro* and *in vivo* investigations suggest that insulin and insulin-like growth factors (IGFs) may have a role in endometrial carcinogenesis, acting synergistically with estrogens [66]. Women with high-risk factors, especially those who had previous breast or

endometrial cancer, represent a target for therapeutic intervention. By reducing estrogen levels, Als proved to be effective, even more than tamoxifen, in decreasing the long-term recurrence of estrogen-dependent cancer and the related mortality rate [67]. However, the benefits of treatments with Als for extended periods must be carefully evaluated in light of the possible negative effects of hypoestrogenic statuses, especially on bone health. On that basis, DCI may have a potential application for a long-term modulation of aromatases activity after Als termination, particularly in obese women with increased activity of aromatases even in the postmenopausal period [68].

4.6. Polycystic ovary syndrome

PCOS is generally characterized by insulin resistance, which entails a reduced MI-to-DCI conversion in most organs [69, 70]. The ovaries represent an exception because they never develop low insulin sensitivity, resulting in ovarian hyperinsulinemia [71]. As a consequence, PCOS patients show an altered granulosa-to-theca layer ratio in the follicles, with the theca layer being anomalously thicker than granulosa [72]. Hyperinsulinemia increases the LH signal on theca cells, boosting the biosynthesis of testosterone and leading to a hyper-estrogenic state [73]. Moreover, hyperinsulinemia in PCOS women leads to increased MI to DCI conversion and to decreased MI/DCI ratios in theca cells [8] and follicular fluid [74]. In a recent review [69], Genazzani concluded that a tailored treatment with MI and DCI should be advisable to restore the altered inositol ratio in the ovaries of PCOS women. In particular, supplementation with MI and DCI, combined in the 40:1 physiological ratio [75, 76, 77, 78], has been found to improve the phenotype and the fertility profile of such patients. However, administration of DCI to insulin-resistant PCOS patients may prove beneficial and reduce the systemic insulin levels only in the short term; and longer treatment with DCI may lead to reduced expression of aromatase, with the potential increase of testosterone levels [16] and the consequent worsening of PCOS symptomatology. The available evidence on PCOS women suggests that the effect of DCI on aromatases may be a further explanation of the lower effectiveness of DCI alone supplementation in these patients.

4.7. Senile hypogonadism and male infertility

The pathological decreased levels of circulating testosterone in men can alter physical characteristics and the reproductive function. The condition leads to reduced libido, erectile dysfunction, infertility, lack of energy, reduced lean muscle mass with associated body fat gain, gynecomastia, impaired cognition, and psychological problems [79]. Hypogonadism may arise from testicular diseases (primary) or from the dysfunction of the hypothalamic-pituitary-gonadal axis (secondary). Hormonal imbalance in men over 40 years may bring to reduced production of gonadotropins, LH in particular, and to the onset of secondary hypogonadism (known as late-onset hypogonadism) [80]. Als proved to decrease the negative feedback of estrogens on the hypothalamus and stimulate the release of endogenous gonadotropins. In particular, treatments with Als seem to ameliorate the hormonal status and to reduce both mastalgia and gynecomastia [81], ameliorating the clinical condition of men with secondary hypogonadism. In this view, whether DCI's action as an inhibitor of aromatase should be confirmed, this compound could be evaluated as a novel potential treatment strategy for male hypogonadism and related subfertility, aiming to increase androgen levels, particularly in men with obese-related secondary hypogonadism [82].

5. Safety

To the best of our knowledge, specific data concerning the safety of DCI are unavailable. The lack of studies that consider or specifically investigate the topic prevents to set boundaries to the therapeutic profile. Based on the aforementioned studies on DCI, we can confidently infer that the administration of DCI up to 1-2 g/day, even for extended periods of time, is safe and devoid of relevant side effects. However, the intake of DCI over this threshold may influence the metabolism of steroid hormones, altering the physiologic menstrual cycle. The activity on aromatase, in particular, may possibly cause an increase of androgen levels and the consequent worsening of the symptoms in women with PCOS.

6. Conclusions

Besides carrying the intracellular message of insulin, DCI influences the steroid biosynthesis by inducing the testosterone production and modulating the aromatase activity. Such an effect on aromatase may be exploited in the treatment and prevention of all the conditions that are generally treated with AIs and/or insulin sensitizers. The use of DCI prevents the build-up of a hypo-estrogenic environment, avoiding the onset of side effects generally associated with therapies with AIs.

7. Expert opinion

Accumulating evidence suggests that biologically active metabolites of DCI are deeply involved in modulating steroidogenesis, even though the exact underlying mechanisms remain to be elucidated. In particular recent data proved that DCI, besides being one of the intracellular second messengers of insulin, affects the activity of aromatase and regulates the androgen-to-estrogen ratio. The mechanistic insights lately reported by Sacchi et al. demonstrated that DCI downregulates the gene expression of the enzyme [17], rather than inhibiting its activity. Hence, DCI modulates the levels of estrogens, and DCI treatment may reduce them without completely blocking their biosynthesis. In a recent publication, we speculated about a possible similar therapeutic role of DCI compared to AIs [18], even though the effects are markedly different and DCI administration avoids the onset of hypo-estrogenic states. Because of such an advantage over AIs, investigating the potentialities of DCI treatment for estrogen-dependent conditions is particularly appealing. In our opinion, future studies should be planned to assess the potential therapeutic effect of DCI administration on the symptoms of gynecological benign conditions such as endometriosis and uterine fibroids, as well as the preventive activity on pre-cancerous lesions. Likewise, DCI treatment should be evaluated as an adjuvant strategy to AIs in the post-operative management of estrogen-sensitive tumors, or as a prevention protocol for subjects presenting with risk factors. Specifically, obese women, particularly in menopause, may benefit from a double advantage of the long-term supplement of DCI due to the effects on insulin resistance and to the modulation of the activity of the aromatase in adipose tissue. Nevertheless, particularly in fertile age, we take the opportunity to underline that long-term treatments with elevated doses of DCI may cause an unwanted increase in testosterone levels as a secondary effect. Such occurrence should be considered in evaluating the therapeutic approach with DCI, especially for those pathological conditions whose symptoms may be exacerbated by increased androgen levels, such as PCOS [16]: in these cases, we believe that the mechanism of action fails to support a rationale for the use of high doses of DCI alone as successful treatment.

DCI is an insulin-sensitizing agent, and short-term supplementation may be beneficial to treat hyper-androgenic or hyper-estrogenic states associated with hyper-insulinemia. Nevertheless, the effect of DCI on aromatase can further explain why short-term treatments with DCI successfully induced ovulation in a dose-dependent manner in PCOS patients [28]. DCI is able to reduce the negative feedback on gonadotropin release not only by reducing systemic insulin levels but even reducing estrogen production. On that basis, we suggest that a similar effect may potentially be observed also in non-PCOS, non-IR anovulatory women, which may benefit from an initially increased FSH release by reduced estrogens. Thus, evaluating the possibility of high-doses supplementation with DCI and the duration of the treatment would be an interesting option in such patients.

Interestingly, hypogonadal men can benefit from an increase of testosterone, which is unwanted in women. They, indeed, exhibit low testosterone levels and altered androgen-to-estrogen ratios, suffering from reduced fertility and altered physical characteristics. Treatment with DCI, by reducing the androgen-to-estrogen conversion, may help to decrease the negative feedback of estradiol on the hypothalamus also in this case, stimulating the release of endogenous gonadotropins and improving the clinical condition of men with secondary hypogonadism. Particularly in men with obesity-related hypogonadism, the long-term supplement of DCI may be useful for both to the effects on insulin resistance and on the activity of the aromatase in adipose tissue [82, 83, 84].

Based on the available evidence, we suggest that the administration of DCI should be carefully considered in light of the patient's specific situation (the type of condition, insulin status, gender), evaluating the proper dosage and treatment duration accordingly. Further studies based on DCI administration should aim to define the safety profile and the therapeutic rationale for each specific condition, such as estrogen-

dependent gynecological pathologies (endometriosis, uterine fibroids) as well as high estrogen production in obese male and female patients, a possible target of DCI supplementation. In this scenario, potential hypo-estrogenic state caused by high levels of DCI may play a positive role to counteract the typical pro-estrogenic microenvironment associated with the progression of endometriosis and enlargement of fibroids. Nevertheless, targeted trials will be mandatory in order to define the best dose/duration of DCI therapy for each disease, considering the patients' condition.

Funding

This paper was not funded

Declaration of interest

V Unfer is an employee at Lo.Li Pharma s.r.l., Rome, Italy. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Articles of special interest have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. Unfer V, Facchinetti F. Editorial - Update on Inositol(s). *Eur Rev Med Pharmacol Sci.* 2017;21(2 Suppl):1-3.
2. Facchinetti F, Appetecchia M, Aragona C, et al. Experts' opinion on inositols in treating polycystic ovary syndrome and non-insulin dependent diabetes mellitus: a further help for human reproduction and beyond. *Expert Opin Drug Metab Toxicol.* 2020;16(3):255-274.
**** This Expert Opinion provides a detailed overview of the characteristics of MI and DCI, and some of their most recent therapeutic applications.**
3. Papaleo E, Unfer V, Baillargeon JP, et al. Contribution of myo-inositol to reproduction. *Eur J Obstet Gynecol Reprod Biol.* 2009;147(2):120-3.
4. Unfer V, Forte G. Does inositol ratio orchestrate the fate of ovarian follicles? *Med Hypotheses.* 2020;144:109983. doi: <https://doi.org/10.1016/j.mehy.2020.109983>.
5. Sun TH, Heimark DB, Nguyen 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(3):1092-8.
6. Facchinetti F, Espinola MSB, Dewailly D, et al. Breakthroughs in the Use of Inositols for Assisted Reproductive Treatment (ART). *Trends Endocrinol Metab.* 2020. doi: 10.1016/j.tem.2020.04.003.
7. 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. In: Genazzani A., Tarlatzis B. (eds) *Frontiers in Gynecological Endocrinology. ISGE Series.* 2016: Springer, Cham.
8. 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(2):111-7.
9. 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(7):854-858.
10. Bevilacqua A, Dragotto J, Giuliani A, et al. Myo-inositol and D-chiro-inositol (40:1) reverse histological and functional features of polycystic ovary syndrome in a mouse model. *J Cell Physiol.* 2019;234(6):9387-9398.
11. Ravanos K, Monasta G, Pavlidou T, et al. Can high levels of D-chiro-inositol in follicular fluid exert detrimental effects on blastocyst quality? *Eur Rev Med Pharmacol Sci.* 2017;21(23):5491-5498.
12. Nestler JE, Romero G, Huang LC, et al. Insulin mediators are the signal transduction system responsible for insulin's actions on human placental steroidogenesis. *Endocrinology.* 1991;129(6):2951-6.
13. 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.
14. Nestler JE, Jakubowicz DJ, de Vargis AF, et al. Insulin stimulates testosterone biosynthesis by human thecal 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(6):2001-2005.
15. Nestler JE. Regulation of the aromatase activity of human placental cytotrophoblasts by insulin, insulin-like growth factor-I, and -II. *TJ Steroid Biochem Mol Biol.* 1993;44(4-6):449-457.
*** This study proved that a DCI-based synthetic phosphoglycan enhances testosterone biosynthesis in human thecal cells, supporting the idea that DCI may also independently regulate steroidogenesis.**
16. 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(10):1390-7.
*** This study highlighted that administration of elevated doses of DCI increased testosterone levels in PCOS women.**

17. Sacchi S, Marinaro F, Tondelli D, et al. Modulation of gonadotrophin induced steroidogenic enzymes in granulosa cells by d-chiroinositol. *Reprod Biol Endocrinol*. 2016;14(1):52.
**** This paper provides an unprecedented insight into the regulatory mechanism that DCI exerts on aromatase in vitro, demonstrating that DCI reduces the gene expression of the enzyme in a dose response manner.**
18. Lagana AS, Unfer V. D-Chiro-Inositol's action as aromatase inhibitor: rationale and potential clinical targets. *Eur Rev Med Pharmacol Sci*. 2019;23(24):10575-10576.
19. Vestergaard P. Drugs Causing Bone Loss. *Handb Exp Pharmacol*. 2019. doi: 10.1007/164_2019_340.
20. Artini PG, Obino ME, Micelli E, et al. Effect of d-chiro-inositol and alpha-lipoic acid combination on COH outcomes in overweight/obese PCOS women. *Gynecol Endocrinol*. 2020. doi: 10.1080/09513590.2020.1737007.
21. Laganà AS, Barbaro L, Pizzo A. Evaluation of ovarian function and metabolic factors in women affected by polycystic ovary syndrome after treatment with D-Chiro-Inositol. *Arch Gynecol Obstet*. 2015;291(5):1181-6.
22. Balen AH, Morley LC, Misso M, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. *Hum Reprod Update*. 2016;22(6):687-708.
23. Niethammer B, Körner C, Schmidmayr M, et al. Non-reproductive Effects of Anovulation: Bone Metabolism in the Luteal Phase of Premenopausal Women Differs between Ovulatory and Anovulatory Cycles. *Geburtshilfe Frauenheilkd*. 2015;75(12):1250-1257.
24. Li D, Hitchcock CL, Barr SI, et al. Negative spinal bone mineral density changes and subclinical ovulatory disturbances--prospective data in healthy premenopausal women with regular menstrual cycles. *Epidemiol Rev*. 2014;36:137-47.
25. Laganà AS, Rossetti P, Buscema M, et al. Metabolism and Ovarian Function in PCOS Women: A Therapeutic Approach with Inositols. *Int J Endocrinol*. 2016;2016:6306410-6306410.
26. Shaw ND, Histed SN, Srouji SS, et al. Estrogen negative feedback on gonadotropin secretion: evidence for a direct pituitary effect in women. *J Clin Endocrinol Metab*. 2010;95(4):1955-1961.
27. 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(17):1314-20.
28. Nestler JE, Gunn R, Bates S, et al. D-chiro-inositol (INS-1) enhances ovulatory rate in hyperandrogenemic, oligomenorrheic women with the polycystic ovary syndrome. *Fertil Steril*. 2001;76(3):S110-S111.
29. Lagana AS, Garzon S, Casarin J, et al. Inositol in Polycystic Ovary Syndrome: Restoring Fertility through a Pathophysiology-Based Approach. *Trends Endocrinol Metab*. 2018;29(11):768-780.
30. 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(6):417-23.
31. Paul C, Laganà AS, Maniglio P, et al. Inositol's and other nutraceuticals' synergistic actions counteract insulin resistance in polycystic ovarian syndrome and metabolic syndrome: state-of-the-art and future perspectives. *Gynecol Endocrinol*. 2016;32(6):431-438.
32. La Marca A, Morgante G, Palumbo M, et al. Insulin-lowering treatment reduces aromatase activity in response to follicle-stimulating hormone in women with polycystic ovary syndrome. *Fertil Steril*. 2002;78(6):1234-1239.
33. De Leo V, la Marca A, Ditto A, et al. Effects of metformin on gonadotropin-induced ovulation in women with polycystic ovary syndrome. *Fertil Steril*. 1999;72(2):282-285.
34. Sohrabvand F, Ansari S, Bagheri M. Efficacy of combined metformin–letrozole in comparison with metformin–clomiphene citrate in clomiphene-resistant infertile women with polycystic ovarian disease. *Hum Reprod*. 2006;21(6):1432-1435.
35. Blumenfeld Z. The Ovarian Hyperstimulation Syndrome. *Vitam Horm*. 2018;107:423-451.
36. Abramov Y, Barak V, Nisman B, et al. Vascular endothelial growth factor plasma levels correlate to the clinical picture in severe ovarian hyperstimulation syndrome. *Fertil Steril*. 1997;67(2):261-265.
37. Jellad S, Haj Hassine A, Basly M, et al. Vascular endothelial growth factor antagonist reduces the early onset and the severity of ovarian hyperstimulation syndrome. *J Gynecol Obstet Hum Reprod*. 2017;46(1):87-91.

38. Practice Committee of the American Society for Reproductive Medicine. Prevention and treatment of moderate and severe ovarian hyperstimulation syndrome: a guideline. *Fertil Steril*. 2016;106(7):1634-1647.
39. Abramov Y, Elchalal U, Schenker JG. Obstetric outcome of in vitro fertilized pregnancies complicated by severe ovarian hyperstimulation syndrome: a multicenter study. *Fertil Steril*. 1998;70(6):1070-1076.
40. Aboulghar M. Prediction of ovarian hyperstimulation syndrome (OHSS): Estradiol level has an important role in the prediction of OHSS. *Hum Reprod*. 2003;18(6):1140-1141.
41. Nelson SM. Prevention and management of ovarian hyperstimulation syndrome. *Thromb Res*. 2017;151 Suppl 1:S61-S64.
42. Mai Q, Hu X, Yang G, et al. Effect of letrozole on moderate and severe early-onset ovarian hyperstimulation syndrome in high-risk women: a prospective randomized trial. *Am J Obstet Gynecol*. 2017;216(1):42.e1-42.e10.
43. Khattab S, Fotouh IA, Mohees IA, et al. Use of metformin for prevention of ovarian hyperstimulation syndrome: a novel approach. *Reprod Biomed Online*. 2006;13(2):194-197.
44. Abushahin F, Goldman KN, Barbieri E, et al. Aromatase inhibition for refractory endometriosis-related chronic pelvic pain. *Fertil Steril*. 2011;96(4):939-42.
45. Laganà AS, Vergara D, Favilli A, et al. Epigenetic and genetic landscape of uterine leiomyomas: a current view over a common gynecological disease. *Arch Gynecol Obstet*. 2017;296(5):855-867.
46. Laganà AS, Alonso Pacheco L, Tinelli A, et al. Management of Asymptomatic Submucous Myomas in Women of Reproductive Age: A Consensus Statement from the Global Congress on Hysteroscopy Scientific Committee. *J Minim Invasive Gynecol*. 2019;26(3):381-383.
47. Vitale SG, Sapia F, Rapisarda AMC, et al. Hysteroscopic Morcellation of Submucous Myomas: A Systematic Review. *Biomed Res Int*. 2017;2017:6848250-6848250.
48. Bulun SE, Simpson ER, Word RA. Expression of the CYP19 gene and its product aromatase cytochrome P450 in human uterine leiomyoma tissues and cells in culture. *J Clin Endocrinol Metab*. 1994;78(3):736-43.
49. Shozu M, Sumitani H, Segawa T, et al. Overexpression of aromatase P450 in leiomyoma tissue is driven primarily through promoter I.4 of the aromatase P450 gene (CYP19). *J Clin Endocrinol Metab*. 2002;87(6):2540-8.
50. Sinai Talaulikar V. Medical therapy for fibroids: An overview. *Best Pract Res Clin Obstet Gynaecol*. 2018;46:48-56.
51. Giudice LC, Kao LC. Endometriosis. *Lancet*. 2004;364(9447):1789-99.
52. Noble LS, Simpson ER, Johns A, et al. Aromatase expression in endometriosis. *J Clin Endocrinol Metab*. 1996;81(1):174-9.
53. Kitawaki J, Noguchi T, Amatsu T, et al. Expression of aromatase cytochrome P450 protein and messenger ribonucleic acid in human endometriotic and adenomyotic tissues but not in normal endometrium. *Biol Reprod*. 1997;57(3):514-9.
54. Hudelist G, Czerwenka K, Keckstein J, et al. Expression of aromatase and estrogen sulfotransferase in eutopic and ectopic endometrium: evidence for unbalanced estradiol production in endometriosis. *Reprod Sci*. 2007;14(8):798-805.
55. Vetrovicka V, Laganà AS, Salmeri FM, et al. Regulation of apoptotic pathways during endometriosis: from the molecular basis to the future perspectives. *Arch Gynecol Obstet*. 2016;294(5):897-904.
56. Laganà AS, Garzon S, Götte M, et al. The Pathogenesis of Endometriosis: Molecular and Cell Biology Insights. *Int J Mol Sci*. 2019;20(22):5615.
57. Nothnick WB. The emerging use of aromatase inhibitors for endometriosis treatment. *Reprod Biol Endocrinol*. 2011;9:87-87.
58. Mousa NA, Bedaiwy MA, Casper RF. Aromatase inhibitors in the treatment of severe endometriosis. *Obstet Gynecol*. 2007;109(6):1421-3.
59. Remorgida V, Abbamonte LH, Ragni N, et al. Letrozole and desogestrel-only contraceptive pill for the treatment of stage IV endometriosis. *Aust N Z J Obstet Gynaecol*. 2007;47(3):222-5.
60. Bilotas M, Meresman G, Stella I, et al. Effect of aromatase inhibitors on ectopic endometrial growth and peritoneal environment in a mouse model of endometriosis. *Fertil Steril*. 2010;93(8):2513-8.

61. Ferrero S, Evangelisti G, Barra F. Current and emerging treatment options for endometriosis. *Expert Opin Pharmacother*. 2018;19(10):1109-1125.
62. Oner G, Ozcelik B, Ozgun MT, et al. The effects of metformin and letrozole on endometriosis and comparison of the two treatment agents in a rat model. *Hum Reprod*. 2010;25(4):932-937.
63. Yilmaz B, Sucak A, Kilic S, et al. Metformin regresses endometriotic implants in rats by improving implant levels of superoxide dismutase, vascular endothelial growth factor, tissue inhibitor of metalloproteinase-2, and matrix metalloproteinase-9. *Am J Obstet Gynecol*. 2010;202(4):368.e1-8.
64. Huang B, Warner M, Gustafsson JA. Estrogen receptors in breast carcinogenesis and endocrine therapy. *Mol Cell Endocrinol*. 2015;418 Pt 3:240-4.
65. Trabert B, Coburn SB, Falk RT, et al. Circulating estrogens and postmenopausal ovarian and endometrial cancer risk among current hormone users in the Women's Health Initiative Observational Study. *Cancer Causes Control*. 2019;30(11):1201-1211.
66. Tian W, Teng F, Zhao J, et al. Estrogen and insulin synergistically promote type 1 endometrial cancer progression. *Cancer Biol Ther*. 2017;18(12):1000-1010.
67. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet*. 2015;386(10001):1341-1352.
68. Bhardwaj P, Au CC, Benito-Martin A, et al. Estrogens and breast cancer: Mechanisms involved in obesity-related development, growth and progression. *J Steroid Biochem Mol Biol*. 2019;189:161-170.
69. Genazzani AD. Inositol as putative integrative treatment for PCOS. *Reprod Biomed Online*. 2016;33(6):770-780.
70. Genazzani AD, Prati A, Marchini F, et al. Differential insulin response to oral glucose tolerance test (OGTT) in overweight/obese polycystic ovary syndrome patients undergoing to myo-inositol (MYO), alpha lipoic acid (ALA), or combination of both. *Gynecol Endocrinol*. 2019;35(12):1088-1093.
71. Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. *Fertil Steril*. 2011;95(8):2515-6.
72. Cadagan D, Khan R, Amer S. Thecal cell sensitivity to luteinizing hormone and insulin in polycystic ovarian syndrome. *Reprod Biol*. 2016;16(1):53-60.
73. Azziz R, Carmina E, Chen Z, et al. Polycystic ovary syndrome. *Nat Rev Dis Primers*. 2016;2:16057-16057.
74. 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 Jul;21(7):854-858.
75. Monastra G, Unfer V, Harrath AH, et al. Combining treatment with myo-inositol and D-chiro-inositol (40: 1) is effective in restoring ovary function and metabolic balance in PCOS patients. *Gynecol Endocrinol*. 2017;33(1):1-9.
76. 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(5):623-631.
77. Garzon S, Lagana AS, Monastra G. Risk of reduced intestinal absorption of myo-inositol caused by D-chiro-inositol or by glucose transporter inhibitors. *Expert Opin Drug Metab Toxicol*. 2019;15(9):697-703.
78. Facchinetti F, Unfer V, Dewailly D, et al. Inositols in Polycystic Ovary Syndrome: An Overview on the Advances. *Trends Endocrinol Metab*. 2020;31(6):435-447.
79. Rizk PJ, Kohn TP, Pastuszak AW, et al. Testosterone therapy improves erectile function and libido in hypogonadal men. *Curr Opin Urol*. 2017;27(6):511-515.
80. Dudek P, Kozakowski J, Zgliczyński W. Late-onset hypogonadism. *Prz Menopauzalny*. 2017;16(2):66-69.
81. Tan RBW, Guay AT, Hellstrom WJG. Clinical Use of Aromatase Inhibitors in Adult Males. *Sex Med Rev*. 2014;2(2):79-90.
82. Fernandez CJ, Chacko EC, Pappachan JM. Male Obesity-related Secondary Hypogonadism - Pathophysiology, Clinical Implications and Management. *Eur Endocrinol*. 2019;15(2):83-90.
83. Rambhatla A, Mills JN, Rajfer J. The Role of Estrogen Modulators in Male Hypogonadism and Infertility. *Rev Urol*. 2016 2016;18(2):66-72.

84. Carrasquillo R, Chu K, Ramasamy R. Novel Therapy for Male Hypogonadism. *Curr Urol Rep.* 2018;19(8):63-63.

ACCEPTED MANUSCRIPT