European Review for Medical and Pharmacological Sciences 2021; 25: 4799-4809

The relevance of inositols treatment for PCOS before and during ART

P.G. ARTINI[1], E. MALACARNE[1], V. TOMATIS[2], A.D. GENAZZANI[2]

[1]Department of Experimental and Clinical Medicine, Division of Obstetrics and Gynecology, University of Pisa, Pisa, Italy
[2]Gynecological Endocrinology Center, Department of Obstetrics and Gynecology, University of Modena and Reggio Emilia, Modena, Italy

Abstract. – Polycystic ovary syndrome (PCOS) is an endocrine disorder that occurs in 8-10% of women of reproductive age. It is characterized by oligo or anovulation, hyperandrogenism and/or polycystic ovaries, but also by an increased insulin plasma level especially in overweight/obese women or in those with familial diabetes. In the last years, among the insulin sensitizers, the use of the two active isoforms of inositols (myo-inositol and d-chiro-inositol) has been spreading for the treatment of PCOS insulin resistance. Several studies have shown a positive role of inositols both on the metabolic profile of PCOS patients, but also on hormonal parameters. Hence, inositols can positively affect the infertility that characterizes many PCOS patients, acting both on ovarian function and spontaneous ovulation and during IVF procedures, in terms of oocyte quality and pregnancy rate.

Key Words:
Polycystic ovary syndrone, PCOS, Inositols, ART myo-inositol, D.chiro-inositol.

PCOS as an Endocrine and Dysmetabolic Disease

Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder that a gynecologist may face in his office. Usually PCOS occurs in 8-10% of women of reproductive age[1,2] and due to the multiple heterogeneity of the syndrome[3] there has been no agreement on the criteria on which to base the diagnosis of PCOS.

At the beginning, diagnostic criteria proposed by the NIH for PCOS were the presence of hyperandrogenism and chronic anovulation with clear exclusion of related ovulatory or other androgen excess disorders (i.e., hyperprolactinemia, thyroid diseases, androgen-secreting tumors and adrenal dysfunction/hyperplasia)[4]. These criteria did not include the presence of polycystic ovaries at ultrasound examination because the polycystic ovaries could also be found in healthy eumenorrheic women[5]. A few years later, the diagnostic criteria were expanded and PCOS was considered as present when at least two of three features were diagnosed: oligo or anovulation, clinical/biochemical hyperandrogenism and polycystic ovaries as assessed by ultrasound examination[6]. This evolution was relevant because it permitted the inclusion of women with PCOS who were excluded by previous criteria: those with polycystic ovaries affected by hyperandrogenism and ovulatory cycles, or chronic anovulation and normal androgen levels. After assessing this, we have then to clarify that PCOS is completely different from PCO. PCO means polycystic ovary and refers only to the morphological aspect of the ovary at ultrasound examination. Indeed, PCO can be found in many other endocrine diseases, such as hyperprolactinemia, thyroid dysfunction, stress-induced amenorrhea. As a major feature, in this last decade, a metabolic aspect has been introduced and taken in account to better approach not only the diagnosis but also the therapeutic choice, that is insulin resistance (IR) and the induced compensatory hyperinsulinemia.

Endocrine Profile of PCOS

PCOS is classically characterized by elevated plasma concentrations of androgens, from ovarian and adrenal origin, increased luteinizing hormone (LH) and estrogen levels (mainly estrone produced by extra glandular conversion from androgens), reduced sex hormone-binding globulin (SHBG) and increased prolactin and insulin levels, the latter often in combination with overweight or obesity[2]. Although PCOS has been recognized to have a controversial origin[7-9], PCOS is classically marked by elevated LH and normal or relatively

Corresponding Author: Paolo Giovanni Artini, MD; e-mail: pgartini@gmail.com

low Follicle stimulating hormone (FSH) secretion so that to show a high LH:FSH ratio (>2.5)[7,8], a higher LH response to GnRH stimulation test[7,8] and an increased LH pulse frequency[4,7,10] that is at the basis of a greater response of theca cells with a subsequent excess of androgen production/secretion and an impaired follicular development[4].

Excess of androgens is classical of the syndrome, although it is not constant[7] and it is mainly due to ovarian production with an adrenal contribution. A certain percentage of PCOS patients might have a mild steroidogenetic defect of adrenal enzyme(s) (such as for 21-hydroxylase) or a relative adrenal hyper-activation due to stress[11]. Androstenedione and testosterone are mostly produced by the ovary (being testosterone derived also from peripheral conversion of androstenedione) while dehydroepiandrosterone sulphate (DHEAS) is the best marker of adrenal secretion. Since cytochrome p450c17 is the androgen-forming enzyme in both the adrenal glands and the ovaries, whatever impairment occurs at the adrenal gland or whatever change occurs on insulin sensitivity (i.e., the increase of insulin resistance with compensatory hyperinsulinemia) triggers the increase of androgen secretion favoring the hyperandrogenism of PCOS[4]. In addition, any excess of testosterone is converted by 5α-reductase to dihydrotestosterone (DHT) determining an excessive androgenic milieu that in the skin might induce the presence hirsutism[12]. Additionally, plasma levels of estrone, a weak estrogen 100 times less bioactive than estradiol, are increased due to the aromatase activity – more active in PCOS than in healthy controls – that transforms androstenedione to estrogens. These events predispose to a chronic hyperestrogenic state with a change of the rate of transformation of estradiol to estrone thus predisposing to a higher risk of endometrial proliferation and to a possible increased risk for endometrial cancer[13-15]. Important to note is the fact that the hyperandrogenic state reduces the synthesis of SHBG determining a higher grade of unbound circulating steroids (mainly androgens) thus triggering the occurrence of hirsutism and acne[4].

Insulin Resistance (IR) and Compensatory Hyperinsulinism

The presence of increased insulin plasma level is the new clinical feature that can be frequently observed in PCOS patients, especially in combination with overweight or obesity. Indeed, overweight/obesity represent a very frequent occurrence in up to 50-70% of PCOS and most often they both occur when there is the presence of familiar diabetes. The occurrence of diabetes in first grade relatives (parents and/or grandparents) is a risk factor not only for the occurrence of IR but mainly for the high percentage of risk of occurrence of gestational diabetes and diabetes in late adulthood[16].

A detailed anamnestic investigation has always to be done to investigate not only the presence of a familial diabetes, but also the fact that the PCOS patients might be born as small for gestational age (SGA) and/or as after a IUGR (Intra Uterine Growth Retardation) or may be born after a pregnancy during which a gestational diabetes occurred[17,18]. As previously reported, such events predispose and trigger IR and predispose to PCOS[18].

Such kind of background(s) are at the basis of the occurrence of insulin resistance as a perfect combination of genetic and epigenetic factors that might be able to trigger the onset of a compensatory hyperinsulinemia[18]. The presence of familiar diabetes predisposes at a higher extent to a less efficient post-receptor signalling driven by inositols non only for the insulin-induced glucose control but also for FSH action on granulosa cells and for TSH stimulus on thyroid cells[16,19]. As additional fact, alpha lipoic acid (ALA), a potent insulin sensitizer produced by mitochondrion, is abnormally synthetized in case of familiar diabetes[20,21].

As additional fact, an excess of androgen milieu may determine abnormalities in glucose metabolism, since it can be an additional cause of abnormal insulin sensitivity. In fact, androgens may directly reduce peripheral and hepatic insulin action. Indeed, testosterone is known to increase IR in PCOS patients since it acts on the post-binding signal reducing the number and efficiency of type 4 glucose transporter (GLUT-4), both in muscle and fat tissues[22]. Moreover, women with central obesity, easily observed in obese PCOS, have higher free androgen levels and show higher levels of IR when compared with weight-matched controls[4].

How to Manage PCOS and the Compensatory Hyperinsulinemia?

The real target for physicians is to reduce and avoid the great risks that PCOS patients have due to such a disease. Though anovulation and/or hyperandrogenism impair reproductive ability, they are not the only impairments a PCOS patient might face. The great deal is to blunt the mainte-

nance of such combination so that to avoid that any genetic and/or epigenetically induced risks might create a more severe predisposition to other diseases. The compensatory hyperinsulinemia is the "biological" solution to face this situation and we all know very well that it is a predisposing factor for metabolic syndrome. The lifestyle, the optimal choice of nutrients and food, physical activity are the easiest solutions. If pregnancy is not a target, an estro-progestin pill is the good solution to overcome the hyperandrogenism that most of PCOS patients have. In case the patient wants to achieve pregnancy, the contraceptive solution is avoided, and the administration of insulin-sensitizer drug(s) might be proposed in combination with a drastic lifestyle.

The dangerous aspect of having IR, whatever is the triggering, is to maintain such abnormal condition up to the perimenopausal period when the physiological changes induced by the menopausal transition take place and, among them, there is the physiological increase of the insulin resistance. It is evident that any PCOS patient should improve her metabolic health years before the occurrence of the perimenopausal transition so that to avoid an increased risk of metabolic syndrome up to diabetes and of all cardio-vascular risks.

The lack of ovulation is a frequent complaint of PCOS patients since this syndrome significantly impairs fertility. Usually, the use of the contraceptive pill is discarded but, in some cases, it might be proposed the use for a certain amount of months during which life-style, i.e., diet and physical activity, are modified using also insulin sensitizers, such as metformin[2] and/or inositols and alpha lipoic acid (ALA)[20,23-27]. Body weight reduction is essential to recover a normal ovulatory function but also to avoid triggering a greater pregnant-induced insulin resistance up to gestational diabetes, in case of pregnancy.

A correct lifestyle is fundamental to counteract the severe compensatory hyperinsulinemia that hits approximately 70% of women with PCOS and overweight or central obesity[28]. Interestingly, it occurs also in 15-30% of lean/normal weight women with PCOS[2,29]. This is a clear indication that a built-in predisposition is at the basis of the metabolic impairment[16]. On such basis, insulin sensitizers have been proposed as the putative treatment to blunt such compensatory hyperinsulinemia always together with a change in lifestyle[2,30]. At present, the most widely used drug is metformin at variable dosages depending on the severity of the glucose metabolic impairment[2,31]. The use of metformin does not reduce hyperandrogenism better than oral contraceptive[32], but as recently reported, the typology of PCOS to be treated is of great relevance, since only when insulin sensitivity is abnormal (i.e., compensatory hyperinsulinemia occurs), metformin shows a greater efficacy on all the PCOS features including hyperandrogenism[33]. Other metabolically active hormones, such as leptin, resistin, adiponectin, and ghrelin are positively activated by metformin administration and thus participate in the improvement of the reproductive function at the hypothalamus-pituitary-ovarian level. Though safe at high dosages, such as 1.5-2 gr or higher, metformin induces various gastrointestinal side effects, such as diarrhea, vomiting, nausea thus reducing greatly the compliance[34].

In these last decade new integrative approaches to PCOS insulin resistance have been proposed using various compounds such inositols in the two clinically active isoforms, i.e., myo-inositol (MYO) and d-chiroinositol (DCI)[16], and alpha lipoic acid (ALA)[35,36].

Research has clearly disclosed that the role of inositolphosphoglycan (IPG) mediators of insulin action[37] and growing evidence suggest that a deficiency of D-chiro-inositol (DCI) containing IPG might be at the basis of insulin resistance, very frequent in PCOS patients. In fact, PCOS patients have a reduced urinary excretion of DCI[38] and metformin administration in obese PCOS patients improves the release of DCI-IPG mediator[39]. Such observations disclose the hypothesis that probably an impairment on DCI synthesis takes place in a high percentage of PCOS patients[16]. Indeed, DCI is synthesized by an epimerase that converts myo-inositol into DCI and depending on the specific needs of the two molecules, each tissue/organ has its own typical conversion rate[40]. Being MYO administration able to induce regular menses in both lean and obese hyperinsulinemic PCOS patients[41], a clear modulatory role of MYO on the insulin-mediated endocrine effects has been hypothesized[16,40,41]. It has been suggested that some abnormal action of insulin might be dependent from an impairment of the IPG mediators of insulin action and suggest that a deficiency in a specific DCI-containing IPG may underlie insulin resistance, similarly, to type 2 diabetes.

In fact it has been demonstrated that the urinary excretion of DCI is reduced, in both humans and experimental animals affected by type 2 diabetes, with an increase in MYO urinary content and that this is not due to the diabetic condition

but to an impairment at the basis of the insulin resistance[42,43]; the epimerase that determines MYO to DCI conversion, is insulin dependent and that there is a decreased amount of DCI production in insulin-sensitive tissues/organs, such as the kidney, liver and muscle of experimental animals with insulin resistance[44,45]. In addition, a marked decrease of epimerase bioactivity was demonstrated in these models[46], thus supporting the hypothesis that insulin resistance *per se* is triggered by some kind of abnormal enzymatic expression[16].

On such basis, it is important to know what happens once insulin binds to its own receptor. A specific couple of cascades are activated on the inner surface of the cell membrane. Through the first one, the substrates of the insulin receptor (IRS) activate various proteins such as PI3K (phosphoinositide 3 kinase) and PDK (phosphoinositide-dependent kinase). Through these, PKB/Akt (protein kinase B/Akt) is activated so that to induce glucose transporter 4 (GLUT-4) translocation to the plasma membrane to permit glucose upload[16]. Through the second cascade, the IRS *via* a G protein (Gp) coupled to a phospholipase D (PLD), induces the hydrolysis of a glycosyl phosphatidylinositol (GPI), which releases an inositol phosphoglycan containing D-chiro-inositol, which acts as second messenger of the insulin signal. This phosphoglycan containing D-chiro-inositol specifically induces the storage of glucose as glycogen in the cytoplasm and, at the same time, induces the oxidative use of glucose inside the mitochondria[16]. Such contemporary equilibrated presence of MYO and its transformation (through the epimerase activity) in DCI permits the optimal utilization of glucose as described by Croze et al[47] being MYO the promoter of glucose upload and DCI the one promoting both the oxidative use of glucose is inside the mitochondrion and the glycogen synthesis is in the cytosol.

In fact, while MYO greatly improves the upload of glucose inside the cell, DCI is mainly acting on one side on the storage of glycogen and on the other side on the use of glucose to produce energy inside the mitochondria. It is thus clear that both MYO-IPG and DCI-IPG are needed so that to permit to have a constant change in the glucose concentrations inside the cytosol. Hence, while glucose enters (thanks to MYO), DCI permits its disappearance storing it as glycogen or burning it producing energy[48]. Both these events create a gradient of concentration and glucose upload from the intercellular spaces is granted. It is intuitive the fact that any kind of change of the ability to have an adequate DCI production, might impair the correct performance of the whole mechanism permitting a reduced glycogen and glucose-derived energy production, thus slowing the upload of glucose[48]. At the end such effects leave greater amounts of glucose outside the cell, triggering the increase of insulin secretion and the beginning of the compensatory hyperinsulinemia.

MYO and DCI to Counteract Hyperinsulinemia in PCOS Patients

Some scholars[27] support a specific role for MYO on gonadotropin-induced ovarian function though not confirmed by others[38]. However, a recent review[49] supports as specific integrative approach to PCOS with hyperinsulinemia, supporting the role of MYO administration alone or in combination with DCI[50]. Indeed, MYO administration has been demonstrated to modulate insulin sensitivity in overweight PCOS patients improving all hormonal parameters and improving insulin sensitivity[27,37]. The daily dosage of 2 g in the morning has been reported to be effective in hyperinsulinemic obese PCOS patients with fasting insulin levels above 12 mU/ml[37]. Such insulin level seems to be a putative cut-off that suggests when MYO administration might give higher chances of success not only on hormonal parameters but also on hyperinsulinemia and insulin sensitivity[37]. However, MYO integrative administration has not been so effective when administered alone in PCOS with familial diabetes[51] in comparison to what observed when DCI alone has been administered at the daily dose of 500 mg[25]. Such kind of clinical observation sustains the fact that the presence of familial diabetes is crucial and impairs the natural biological conversion of MYO to DCI through the defect of epimerase expression/activity.

When DCI is administered, both PCOS with or without familial diabetes have been reported to show the improvement of ovarian function and the significant decrease of the hyperandrogenic state[25,52].Such clinical studies support the hypothesis of the presence of a defect in the insulin-signaling pathway mainly related to a reduced expression/synthesis of the epimerase that converts MYO to DCI thus reducing the amount of DCI-PG created. This decrease of the efficiency of the post-receptor insulin signaling contributes and triggers the compensatory hyperinsulinemia and the pathophysiology of the insulin resistance of PCOS, as supposed by Baillargeon et al[38]. On such basis, recently, a combination of both MYO

and DCI has been proposed as a putative integrative approach for impaired metabolism of PCOS. Clinical data suggest that a combination of MYO and DCI at a MYO-to-DCI ratio of 40:1 seems to be the most appropriate to improve both metabolic and reproductive impairment since it resembles the physiological ratio[50,53] while according to Nordio et al[53] the use of DCI alone resulted not effective. Practically speaking this combination is supposed to act on reproduction through MYO and on glucose metabolism through DCI, overcoming the eventual impairment on epimerase expression/function. Apparently, such strategy might be correct but recent data disclose some problems. In fact, recently it has been pointed out that, being inositols chemically and structurally similar to glucose, they compete with glucose for intestinal absorption especially if administered during meals[50,53]. For this reason whatever inositol is prescribed, it has to be taken far from breakfast and lunch. In fact, exactly for this reason, combinations of MYO and DCI, such as the 40:1, might not be greatly effective in PCOS women, since both compete to be absorbed in the intestine[54]. Theoretically, only the administration of higher dosages of both compounds can grant an adequate absorption of both MYO and DCI.

Inositol and IVF Treatments

Several studies[55-57] have shown a fundamental role of MYO on female fertility. MYO regulates several functions as oocyte maturation, fertilization and early embryonic development. In fact MYO administration has been reported to act positively on ovarian function[58] and oocyte quality in patients undergoing IVF procedures[59].

Inositols can positively affect several aspects of PCOS, acting first on insulin resistance and on other metabolic aspects of these patients such as a reduction of total and free testosterone, a lowering of blood pressure and a better control of blood glycaemia. In addition, MYO seems to have also a positive role on modulation of serum androgens and circulating LH and FSH levels, with a beneficial action on the ovulation frequency[52,60-64].

Exactly for these reasons, and for its beneficial role on women fertility, it has been hypothesized to use inositol as adjuvant in IVF treatment during ovarian stimulation with FSH[65]. Given the predominant role of MYO on ovary, its usefulness in IVF cycles was analyzed and many authors stressed its positive role in PCOS women who underwent ovarian stimulation.

In our randomized study (2013)[66] we compared two groups of women who underwent controlled ovarian hyperstimulation for IVF (fertilization *in vitro* embryo-transfer)/ICSI (intracytoplasmic sperm injection) with recombinant FSH and GnRH agonist from mid-luteal phase. One group (25 women) was treated with MYO plus folic acid, while the control group (25 women) was treated only with folic acid, both for 12 weeks before ART. In the MYO group the duration of stimulation was shorter, the dose of r-FSH were lower and estradiol levels evaluated at the day of hCG administration were lower than in control group. Pregnancy rate (bHCG positive) was higher in the MYO treated group. Moreover, in this group only one cycle was cancelled, while four were cancelled in the control group. All these cycles were cancelled because of a risk of Ovarian Hyper-Stimulation Syndrome (OHSS). In fact, many authors highlighted a possible beneficial role of MYO supplementation on the risk of OHSS: low levels of E2 were found in these patients with a consequently reduction of cancelled cycles due to OHSS[62,65]. This study clearly support the relevant role of MYO as second messenger of FSH signal and stimulation on the granulosa cells since the supplementation permitted to significantly reduce the amount of r-FSH used and to obtain significantly better outcomes[66].

A review published in 2017[67], however, showed that an exclusive supplementation with MYO had no positive effects on oocyte maturation, embryo quality or pregnancy rate, denying the previous data. Due to conflicting results, a recent review investigated the role of MYO in PCOS patients, to evaluate its beneficial effects during ART. Authors summarized the main randomized studies in which MYO was used, demonstrating positive effects of MYO on quality and maturation of oocytes, quality of embryos and ovarian function. Different dosages of MYO were used in the studies examined: in six of them the patients of the study group received 4 gr of MYO (alone or in combination), while in a minority of studies the dosage was lower (2 gr or 1.1 gr). Whereas other meta-analyses were unsuccessful to conclude on the positive effect of MYO in PCOS women, the same authors specified that the debate on this topic was still opened: they concluded indicating that the optimal dosage of MYO was 4 gr per day (2 gr twice), starting three months prior to stimulation, but large multicenter randomized controlled trials were required[68].

Piomboni et al[69] analyzed follicular fluid and oocyte quality in PCOS women who underwent

COH, comparing three groups: a group of 26 women treated with D-chiro-inositol, a group of 20 women treated with metformin and an untreated group of 22 women. They started therapy 3 months before ovarian stimulation protocol. They highlighted a higher number of good qualities MII oocytes and a reduction of oxidative stress in follicular fluid in DCI and metformin groups in comparison to untreated group[69]. It was clear that DCI played a fundamental role in these patients, even if the correct dosage to be used to give the best benefits, both on metabolic aspect but above all on the ovary, remains debated[70]. Considering their different actions on the cells and their different concentration in human tissues, it is conceivable that both must be used to obtain the most effective therapeutic effect[64].

Unfer et al[62] suggested that an association of MYO/DCI was essential to improve reproductive outcomes. They showed that MYO had a determinant role in nuclear and cytoplasmic oocyte development. MYO additionally improved oocyte energy status thanks to an increase of glucose uptake it can reduce, during COH, the total dose of FSH necessary for ovarian stimulation. These data were confirmed by a systematic review published in 2018 that showed a statistically significant reduction of total dose of gonadotropins and length of hyperstimulation in PCOS patients who received MYO during IVF[71]. DCI on the other hand acts more on metabolism reducing hyperinsulinemia and acts indirectly on the ovary, although it has been shown that a higher dose can cause some toxicity with harmful effects on the ovary[62]. These data, however, were denied from Mendoza et al[72,73], that in 2019 showed that the administration of a combination of MYO-DCI at high doses of DCI gave better results on pregnancy rate compared to the administration of MYO-DCI at a physiological dosage, without differences in oocyte maturation or embryo quality. These results were subsequently confirmed by another study[73] in which PCOS patients treated with a combination of MYO-DCI with high-DCI concentration (550 mg MYO + 150 mg DCI twice daily; 3.6:1) were compared with PCOS patients treated with a combination of MYO-DCI with low-DCI concentration (550 mg MYO + 13.8 mg DCI twice daily; 40:1). All women underwent controlled ovarian stimulation for ICSI with GnRH antagonist protocol. The results showed that a higher dose of DCI also had beneficial effects on oocytes quality, in particular on the quality of the cytoplasm. According to the authors, the improvement of oocyte quality by DCI could be explained by a direct action on the ovum itself and by an indirect action on the follicular fluid[73].

In recent years, a beneficial role of Alpha-lipoic acid (ALA) has also been highlighted: it is a powerful antioxidant and has been shown to benefit PCOS patients, improving insulin sensitivity and metabolic status[20].

Hyperglycemia indeed can determine an increase in the production of reactive oxygen species (ROS) with a negative impact on several pathways in ovary, including oocyte maturation, ovarian steroidogenesis, corpus luteum functions and development of embryo[74,75]. For this reason, a role of ALA has been hypothesized, in association with inositols, in PCOS patients who underwent *in vitro* fertilization.

In a recent study, infertile non-diabetic overweight/obese patients with PCOS, which underwent treatment with DCI (500 mg) and ALA (300 mg) twice a day before and during COH for IVF/ICSI, were analyzed. A comparison was made between patients with or without diabetic relatives and has been shown as the group of overweight/obese patients with diabetic relatives tended to have a lower dose of gonadotropin, shorter stimulation days, higher number of MII oocytes, and higher number of fertilized oocytes. Although the results are not statistically significant, this work shows us how an accurate anamnestic investigation in PCOS infertile women is imperative to choose the most effective integrative therapeutic strategy during ART[76]. Moreover, recent data focused on the role of genetic predisposition to diabetes disclosing also that in patients with PCOS and familial diabetes, there is also a defect on alpha lipoic acid (ALA) synthesis[21,35] and that also an integration with ALA would be helpful, as recently reported[20]. Such data support the putative use of the combination of MYO or DCI with ALA in counteracting the compensatory hyperinsulinemia in PCOS patients, especially in those PCOS with first grade relatives with diabetes[23,51].

Effects of MYO and DCI on Ovulation Frequency

DCI is a product of the epimerization of the C1 hydroxyl group of MYO and it seems to play a role as a second messenger of insulin and has been seen to be present in higher concentration in fat, muscle and liver, reflecting the different tissue requirement and concentration of NAD/NADH epimerase[77]. Inositols act as second messengers and are fundamental biomolecules in a lot

of signal transduction pathways indispensable for several processes, such us cell membrane formation, lipid synthesis and regulation of hormones activities[77-79]. They are involved in some pathways within ovary and oocytes[80]. MYO, in particular, plays a determinant role in the development of oocyte, modulating the intracellular Ca2+ release[62], and it represents approximately the 99% of the ovarian intracellular pool of inositol[81].

Unfer et al[62] in 2012 published a review in which six RCTs were analyzed. They highlighted the fundamental role of MYO both at a systemic and ovarian level. The main hypothesis was that a supplementation of MYO determined a decrease of insulin levels and HOMA index, maybe by inducing an increase of inositolphosphoglycan (IPG) levels. Furthermore, authors described a reduction of LH levels and LH/FSH ratio after MYO supplementation: in fact, these values are typically increased in PCOS patients. All these elements could explain why supplementation with MYO leads to an improvement of ovulation frequency and it is truly effective in restoring normal menstrual cyclicity[33,82].

It has also been demonstrated that in PCOS patients there is an increase of the epimerase activity in theca cells, with a consequent decrease in MYO reserves. This modification of the regular MYO/DCI ratio in the ovary, can be the basis of the menstrual irregularities and the lack of ovulation[83].

Even though the predominant ovarian role is played by MYO, several authors[84,85] have indicated that a supplementation of both MYO and DCI can determine positive results, acting not only on the ovarian microenvironment, but also quickly restoring the normal hormonal and metabolic parameters. This is most valid in overweight and obese patients, where the metabolic improvement could also provide significant advantages on hormonal and reproductive outcomes. DCI administration has a positive effect on ovulation and ovarian function thanks to its action to reduce hyperinsulinemia and insulin resistance[58,66].

Does Inositol Affect Oocyte Quality?

Many authors in recent years have investigated the possible effect of inositols on oocyte quality in PCOS infertile patients. MYO, acting mainly at the ovarian level, has been reported to have a positive effect on the oocyte in patients undergoing IVF[66].

Unfer et al[86] in 2011 compared forty-three euglycemic PCOS patients who received MYO and forty-one euglycemic PCOS patients who received DCI. All these patients underwent ovulation induction for ICSI. They showed that there was no difference in the total number of oocytes retrieved in the two groups, but the number of mature oocytes was significantly increased in the MYO group. They highlighted also an improvement of the number of top-quality embryos.

In 2013 the positive role of MYO administration was confirmed: even if the total number of oocytes retrieved was lower in the treated group (MYO plus folic acid), these patients had a higher number of top-quality oocytes compared with the control group (folic acid) with percentages of 82% *vs.* 65% (p=0.05)[66]. Other authors have highlighted an increase in the number of metaphase II (MII) oocytes with a simultaneous reduction of degenerated oocytes in PCOS patients treated with MYO during ART procedures[65,87]. Also, the mean number of transferred embryos was higher in the MYO group[87]. Such putative positive role of MYO has been confirmed recently also by Emekçi Özay et al[88].

However, considering the well-established benefit of DCI on ovarian activity and regularity, the possible role on oocyte quality was also analyzed, with conflicting results. Piomboni et al[65] collected samples of follicular fluid after ovarian hyperstimulation in patients who underwent ART, comparing a treated group (metformin or DCI) and a control group. They showed that a higher number of good quality oocytes was observed in DCI or metformin groups in comparison to untreated group, with a statistically significant result[69]. However, some scholars[89] have shown how an increase of concentration of DCI in the follicular fluid could cause a detrimental effect on oocytes quality. Colazingari et al[90] instead compared a group of PCOS patients treated with MYO plus DCI (at the physiological ratio of 1.1 g MYO plus 27.6 mg of DCI) with another group of PCOS patients treated with DCI alone. They underwent ovarian controlled hyperstimulation for IVF-ET and the results obtained showed that only the MYO-DCI therapy was able to increase the quality of oocytes and embryos. In a recent multicenter controlled and randomized study, DCI and MYO were administered to two PCOS groups of patients undergoing ICSI with two different dosages for 12 weeks (study group 550 mg of MYO plus 150 mg of DCI twice daily; control group 550 mg of MYO plus 13.8 mg of DCI twice daily): no differences were found in terms of number of MII oocytes and percentage of good quality em-

bryos[72]. Although some results are conflicting, all these studies certainly show a beneficial role for both MYO and DCI on oocyte quality in infertile PCOS women who underwent ART.

Conclusions and Future Perspectives

On the basis of what discussed up to now, it appears clear that PCOS is a rather complicated endocrinological disease that is greatly dependent from the androgen production (from the ovary and/or from the adrenal gland) but has a clear dependence from a genetic or epigenetic predisposition to hyperinsulinemic triggers, such as a familial diabetes, being born IUGR and/or SGA, having an unbalanced diet favoring amids and/or carbohydrates. All such factors contribute to the increase of insulin production and probably also to a reduced insulin metabolic clearance by the liver thus determining a reactive compensatory hyperinsulinemia that severely affects not only metabolically active organs/tissues, such as liver and fat tissue, but also the reproductive axis.

This scenario clearly requires a specific adaptive and systematic intervention that on one side needs the absolute improvement of the lifestyle (i.e., reduction of the nutrients and food that, once digested, release glucose, greater physical activity) on the other side requires an active intervention. Other than metformin, that often induces many side effects, specific integrative compounds, such as inositols, might increase the physiological amount needed so that to sustain and speed up all the biological pathways that are slowed down by the compensatory hyperinsulinemia. These latter pathways are for sure those that keep reproduction under control.

An adequate and optimal presence of inositols inside the cells of any compartment is for sure at the basis of the perfect function of any organ or tissue. Moreover, being MYO the specific compound required to have an optimal cellular ability not only to respond to insulin (as well as FSH and TSH) linkage to its own receptor, but also to induce the correct DCI production through epimerase activity, it appears clear that both need to be present.

It is remarkably crucial to know whether any predisposing factor(s) exist in inducing a reduced MYO-to-DCI conversion, such as in familial diabetes that impairs also ALA endogenous synthesis. A good anamnestic investigation is fundamental to ascertain together with hormonal and insulin profiles what kind of impairment PCOS patients might have at the basis of the IR. The choice of the integrative inositols treatment is for sure a relevant tool that predisposes to a more receptive ovary to the ovulation induction. Inositols administration clearly improves ovarian response both in terms of oocyte quality, as well as of pregnancy rate. Being inositols natural integrative compounds, they represent a safe and simple treatment to overcome the intrinsic and/or acquired metabolic impairments of PCOS that affects both spontaneous and induced ovarian function.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Carmina E, Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab 1999; 84: 1897-1899.
2) Genazzani AD, Ricchieri F, Lanzoni C. Use of metformin in the treatment of polycystic ovary syndrome. Women's health 2010; 6: 577-593.
3) Carmina E. Genetic and environmental aspect of polycystic ovary syndrome. J Endocrinol Invest 2003; 26: 1151-1159.
4) Zawadzki JK DA. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. Polycystic Ovary Syndrome: Dunaif A, Givens JR, Haseltine FP, Merriam GR (Eds). Blackwell, MA, USA 1992: 337-384.
5) Polson DW, Adams J, Wadsworth J, Franks S. Polycystic ovaries--a common finding in normal women. Lancet (London, England) 1988; 1: 870-872.
6) Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19: 41-47.
7) Hirschberg AL. Polycystic ovary syndrome, obesity and reproductive implications. Womens Health 2009; 5: 529-540; quiz 41-2.
8) Doi SA. Neuroendocrine dysfunction in PCOS: a critique of recent reviews. Clin Med Res 2008; 6: 47-53.
9) Vrbikova J, Hainer V. Obesity and polycystic ovary syndrome. Obes Facts 2009; 2: 26-35.
10) Kalro BN, Loucks TL, Berga SL. Neuromodulation in polycystic ovary syndrome. Obstet Gynecol Clin North Am 2001; 28: 35-62.
11) Genazzani AD, Petraglia F, Pianazzi F, Volpogni C, Genazzani AR. The concomitant release of androstenedione with cortisol and luteinizing hormone pulsatile releases distinguishes adrenal from ovarian hyperandrogenism. Gynecol Endocrinol 1993; 7: 33-41.

12) Plouffe L, Jr. Disorders of excessive hair growth in the adolescent. Obstet Gynecol Clin North Am 2000; 27: 79-99.
13) Vrbikova J, Cibula D. Combined oral contraceptives in the treatment of polycystic ovary syndrome. Human Reprod Update 2005; 11: 277-291.
14) Cibula D, Gompel A, Mueck AO, La Vecchia C, Hannaford PC, Skouby SO, Zikan M, Dusek L. Hormonal contraception and risk of cancer. Human Reprod Update 2010; 16: 631-650.
15) Meczekalski B, Perez-Roncero GR, Lopez-Baena MT, Chedraui P, Pérez-López FR. The polycystic ovary syndrome and gynecological cancer risk. Gynecol Endocrinol 2020; 36: 289-293.
16) Genazzani AD. Inositol as putative integrative treatment for PCOS. Reprod Biomed Online 2016; 33: 770-780.
17) de Melo AS, Dias SV, Cavalli Rde C, Cardoso VC, Bettiol H, Barbieri MA, Ferriani RA, Vieira CS. Pathogenesis of polycystic ovary syndrome: multifactorial assessment from the foetal stage to menopause. Reprod 2015; 150: R11-R24.
18) Ibanez L, Potau N, Francois I, de Zegher F. Precocious pubarche, hyperinsulinism, and ovarian hyperandrogenism in girls: relation to reduced fetal growth. J Clin Endocrinol Metab 1998; 83: 3558-3562.
19) Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 1984; 312: 315-321.
20) Genazzani AD, Shefer K, Della Casa D, Prati A, Napolitano A, Manzo A, Despini G, Simoncini T. Modulatory effects of alpha-lipoic acid (ALA) administration on insulin sensitivity in obese PCOS patients. J Endocrinol Invest 2018; 41: 583-590.
21) Padmalayam I, Hasham S, Saxena U, Pillarisetti S. Lipoic acid synthase (LASY): a novel role in inflammation, mitochondrial function, and insulin resistance. Diabetes 2009; 58: 600-608.
22) Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS. Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. J Clin Endocrinol Metab 1992; 75: 577-583.
23) Genazzani AD, Prati A, Simoncini T, Napolitano A. Modulatory role of D-chiro-inositol and alpha lipoic acid combination on hormonal and metabolic parameters of overweight/obese PCOS patients. Eur Gynecol Obstet 2019; 1: 29-33.
24) Genazzani AD DG, Santagni S, Prati A, Rattighieri E, Chierchia E, Simoncini T. Effects of a combination of alpha lipoic acid and myoinositol on insulin dynamics in overweight/obese patients with PCOS. Endocrinol Metab Synd 2014; 3: 3.
25) Genazzani AD, Santagni S, Rattighieri E, Chierchia E, Despini G, Marini G, Prati A, Simoncini T. Modulatory role of D-chiro-inositol (DCI) on LH and insulin secretion in obese PCOS patients. Gynecol Endocrinol 2014; 30: 438-443.
26) Genazzani AD, Santagni S, Ricchieri F, Campedelli A, Rattighieri E, Chierchia E, Marini G, Despini G, Prati A, Simoncini T. Myo-inositol modulates insulin and luteinizing hormone secretion in normal weight patients with polycystic ovary syndrome. J Obst Gynaecol Res 2014; 40: 1353-1360.
27) Genazzani AD, Prati A, Santagni S, Ricchieri F, Cherchia E, Rattighieri E, Campedelli A, Simoncini T, Artini PG. Differential insulin response to myo-inositol administration in obese polycystic ovary syndrome patients. Gynecol Endocrinol 2012; 28: 969-973.
28) Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev 2011: CD007506.
29) Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, Carmina E, Chang J, Yildiz BO, Laven JS, Boivin J, Petraglia F, Wijeyeratne CN, Norman RJ, Dunaif A, Franks S, Wild RA, Dumesic D, Barnhart K. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril 2012; 97: 28-38 e25.
30) Mario FM, Graff SK, Spritzer PM. Habitual physical activity is associated with improved anthropometric and androgenic profile in PCOS: a cross-sectional study. J Endocr Invest 2017; 40: 377-384.
31) Genazzani AD, Battaglia C, Malavasi B, Strucchi C, Tortolani F, Gamba O. Metformin administration modulates and restores luteinizing hormone spontaneous episodic secretion and ovarian function in nonobese patients with polycystic ovary syndrome. Fertil Steril 2004; 81: 114-119.
32) Cosma M, Swiglo BA, Flynn DN, Kurtz DM, Labella ML, Mullan RJ, Elamin MB, Erwin PJ, Montori VM. Clinical review: Insulin sensitizers for the treatment of hirsutism: a systematic review and metaanalyses of randomized controlled trials. J Clin Endocrinol Metab 2008; 93: 1135-1142.
33) Genazzani AD, Lanzoni C, Ricchieri F, Baraldi E, Casarosa E, Jasonni VM. Metformin administration is more effective when non-obese patients with polycystic ovary syndrome show both hyperandrogenism and hyperinsulinemia. Gynecol Endocrinol 2007; 23: 146-152.
34) Lord JM, Flight IH, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 2003; 327: 951-953.
35) Lee WJ, Song KH, Koh EH, Won JC, Kim HS, Park HS, Kim MS, Kim SW, Lee KU, Park JY. Alpha-lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle. Biochem Biophys Res Commun 2005; 332: 885-891.
36) Shen QW, Zhu MJ, Tong J, Ren J, Du M. Ca2+/calmodulin-dependent protein kinase kinase is involved in AMP-activated protein kinase activation by alpha-lipoic acid in C2C12 myotubes. Am J Physiol Cell Physiol 2007; 293: C1395-1403.
37) 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: 139-144.
38) Baillargeon JP, Diamanti-Kandarakis E, Ostlund RE Jr, Apridonidze T, Iuorno MJ, Nestler JE. Altered D-chiro-inositol urinary clearance in women with polycystic ovary syndrome. Diabetes Care 2006; 29: 300-305.

39) 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: 242-249.
40) Larner J. D-chiro-inositol--its functional role in insulin action and its deficit in insulin resistance. Int J Exp Diabetes Res 2002; 3: 47-60.
41) Genazzani A.D. PA, Despini G., Marini G., Ricchieri F. PCOS from Lifestyle to the Use of Inositol and Insulin Sensitizers. In: Publ. SI, ed. Frontiers in Gynecological Endocrinology, ISGE series 2014: 59-67.
42) Kennington AS, Hill CR, Craig J, Bogardus C, Raz I, Ortmeyer HK, Hansen BC, Romero G, Larner J. Low urinary chiro-inositol excretion in non-insulin-dependent diabetes mellitus. N Eng J Med 1990; 323: 373-378.
43) Ortmeyer HK, Huang LC, Zhang L, Hansen BC, Larner J. Chiroinositol deficiency and insulin resistance. II. Acute effects of D-chiroinositol administration in streptozotocin-diabetic rats, normal rats given a glucose load, and spontaneously insulin-resistant rhesus monkeys. Endocrinology 1993; 132: 646-651.
44) Pak Y, Paule CR, Bao Y, Huang LC, Larner J. Insulin stimulates the biosynthesis of chiro-inositol-containing phospholipids in a rat fibroblast line expressing the human insulin receptor. Proc Natl Acad Sci U S A 1993; 90: 7759-7763.
45) Pak Y, Hong Y, Kim S, Piccariello T, Farese RV, Larner J. In vivo chiro-inositol metabolism in the rat: a defect in chiro-inositol synthesis from myo-inositol and an increased incorporation of chiro-[3H]inositol into phospholipid in the Goto-Kakizaki (G.K) rat. Mol Cells 1998; 8: 301-309.
46) 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: 1092-1098.
47) Croze ML, Soulage CO. Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie 2013; 95: 1811-1827.
48) Genazzani AD. Expert's opinion: integrative treatment with inositols and lipoic acid for insulin resistance of PCOS. Gynecol Repr Endocrinol Metab; 1: 146-157.
49) Milewska EM, Czyzyk A, Meczekalski B, Genazzani AD. Inositol and human reproduction. From cellular metabolism to clinical use. Gynecol Endocrinol 2016; 32: 690-695.
50) Roseff S, Montenegro M. Inositol treatment for PCOS should be science-based and not arbitrary. Int J Endocrinol 2020; 2020: 6461254.
51) Genazzani AD, Prati A, Marchini Petrillo T, Napolitano A, Simoncini T. 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: 1088-1093.
52) 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: 1314-1320.
53) 5Nordio M, Basciani S, Camajani E. The 40:1 myo-inositol/D-chiro-inositol plasma ratio is able to restore ovulation in PCOS patients: comparison with other ratios. Eur Revi Med Pharmacol Sci 2019; 23: 5512-5521.
54) Garzon S, Lagana AS, Monastra G. Risk of reduced intestinal absorption of myo-inositol caused by D-chiroinositol or by glucose transporter inhibitors. Expert Opin Drug Metab Toxicol 2019; 15: 697-703.
55) Downes CP. Twenty-fifth Colworth medal lecture. The cellular functions of myo-inositol. Biochem Soc Trans 1989; 17: 259-268.
56) Downes CP, Macphee CH. myo-inositol metabolites as cellular signals. Eur J Biochem 1990; 193: 1-18.
57) Diaz JR, de las Cagigas A, Rodriguez R. Micronutrient deficiencies in developing and affluent countries. Eur J Clin Nutr 2003; 57 Suppl 1: S70-72.
58) 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: 151-159.
59) Chiu TT, Rogers MS, Law E, 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: 1591-1596.
60) 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: 417-423.
61) Nestler JE, Jakubowicz DJ, Iuorno MJ. Role of inositolphosphoglycan mediators of insulin action in the polycystic ovary syndrome. J Pediatr Endocrinol Metab 2000; 13 Suppl 5: 1295-1298.
62) Unfer V, Carlomagno G, Dante G, Facchinetti F. Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. Gynecol Endocrinol 2012; 28: 509-515.
63) Papaleo E, Unfer V, Baillargeon JP, Chiu TT. Contribution of myo-inositol to reproduction. Eur J Obstet Gynecol Reprod Biol 2009; 147: 120-123.
64) Sortino MA, Salomone S, Carruba MO, Drago F. Polycystic Ovary Syndrome: Insights into the Therapeutic Approach with Inositols. Front Pharmacol 2017; 8: 341.
65) 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: 1750-1754.
66) Artini PG, Di Berardino OM, Papini F, Genazzani AD, Simi G, Ruggiero M, Cela V. Endocrine and clinical effects of myo-inositol administration in polycystic ovary syndrome. A randomized study. Gynecol Endocrinol 2013; 29: 375-379.
67) Mendoza N, Perez L, Simoncini T, Genazzani A. Inositol supplementation in women with polycys-

tic ovary syndrome undergoing intracytoplasmic sperm injection: a systematic review and meta-analysis of randomized controlled trials. Reprod Biomed Online 2017; 35: 529-535.
68) Merviel P, James P, Bouee S, Le Guillou M, Rince C, Nachtergaele C, Kerlan V. Impact of myo-inositol treatment in women with polycystic ovary syndrome in assisted reproductive technologies. Reprod Health 2021; 18: 13.
69) Piomboni P, Focarelli R, Capaldo A et al. Protein modification as oxidative stress marker in follicular fluid from women with polycystic ovary syndrome: the effect of inositol and metformin. J Assisted Reprod Gen 2014; 31: 1269-1276.
70) Bevilacqua A, Dragotto J, Giuliani A, Bizzarri M. 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: 9387-9398.
71) Lagana AS, Vitagliano A, Noventa M, Ambrosini G, D'Anna R. Myo-inositol supplementation reduces the amount of gonadotropins and length of ovarian stimulation in women undergoing IVF: a systematic review and meta-analysis of randomized controlled trials. Arch Gynecol Obstet 2018; 298: 675-684.
72) Mendoza N, Diaz-Ropero MP, Aragon M, Maldonado V, Llaneza P, Lorente J, Mendoza-Tesarik R, Maldonado-Lobon J, Olivares M, Fonolla J. Comparison of the effect of two combinations of myo-inositol and D-chiro-inositol in women with polycystic ovary syndrome undergoing ICSI: a randomized controlled trial. Gynecol Endocrinol 2019; 35: 695-700.
73) Mendoza N, Galan MI, Molina C, Mendoza-Tesarik R, Conde C, Mazheika M, Diaz-Ropero MP, Fonolla J, Tesarik J, Olivares M. High dose of d-chiro-inositol improves oocyte quality in women with polycystic ovary syndrome undergoing ICSI: a randomized controlled trial. Gynecol Endocrinol 2019: 1-4.
74) Gonzalez F, Sia CL, Shepard MK, Rote NS, Minium J. Hyperglycemia-induced oxidative stress is independent of excess abdominal adiposity in normal-weight women with polycystic ovary syndrome. Hum Reprod 2012; 27: 3560-3568.
75) Gonzalez F, Nair KS, Daniels JK, Basal E, Schimke JM, Blair HE. Hyperandrogenism sensitizes leukocytes to hyperglycemia to promote oxidative stress in lean reproductive-age women. J Clin Endocrinol Metab 2012; 97: 2836-2843.
76) Artini PG, Obino MER, Micelli E, Malacarne E, Vacca C, Papini F, Cela V. Effect of d-chiro-inositol and alpha-lipoic acid combination on COH outcomes in overweight/obese PCOS women. Gynecol Endocrinol 2020: 36: 755-759.
77) Unfer V, Porcaro G. Updates on the myo-inositol plus D-chiro-inositol combined therapy in polycystic ovary syndrome. Expert Rev Clin Pharmaco 2014; 7: 623-631.
78) Chen IW, Charalampous CF. Biochemical studies on inositol. IX. D-Inositol 1-phosphate as intermediate in the biosynthesis of inositol from glucose 6-phosphate, and characteristics of two reactions in this biosynthesis. J Biol Chem 1966; 241: 2194-2199.
79) Berridge MJ. Inositol trisphosphate and calcium signalling mechanisms. Biochim Biophys Acta 2009; 1793: 933-940.
80) 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: 408-416.
81) 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: 854-858.
82) Baillargeon JP, Iuorno MJ, Nestler JE. Insulin sensitizers for polycystic ovary syndrome. Clin Obstet Gynecol 2003; 46: 325-340.
83) Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. Fertil Steril 2011; 95: 2515-2516.
84) Unfer V, Nestler JE, Kamenov ZA, Prapas N, Facchinetti F. Effects of Inositol(s) in Women with PCOS: A Systematic Review of Randomized Controlled Trials. Int J Endocrinol 2016; 2016: 1849162.
85) 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: 575-581.
86) 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: 452-457.
87) 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: 509-514.
88) Emekci Ozay O, Ozay AC, Cagliyan E, Okyay RE, Gülekli B. Myo-inositol administration positively effects ovulation induction and intrauterine insemination in patients with polycystic ovary syndrome: a prospective, controlled, randomized trial. Gynecol Endocrinol 2017; 33: 524-528.
89) Ravanos K, Monastra G, Pavlidou T, Goudakou M, Prapas N. Can high levels of D-chiro-inositol in follicular fluid exert detrimental effects on blastocyst quality? Eur Rev Med Pharmacol Sci 2017; 21: 5491-5548.
90) Colazingari S, Treglia M, Najjar R, Bevilacqua A. The combined therapy myo-inositol plus D-chiro-inositol, rather than D-chiro-inositol, is able to improve IVF outcomes: results from a randomized controlled trial. Arch Gynecol Obstet 2013; 288: 1405-1411.