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*Chapter 3*

# STRATEGIES FOR THE TREATMENT OF POLYCYSTIC OVARY SYNDROME (PCOS) WOMEN: THE ROLE OF MYO-INOSITOL (MI) AND D-CHIRO-INOSITOL (DCI) BETWEEN DIET AND THERAPY

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## ABSTRACT

Polycystic Ovary Syndrome (PCOS) is one of the most common female endocrine disorders affecting 6-15% of women in reproductive age. This syndrome involves the hypothalamus, the pituitary gland, the

ovaries and the adrenal gland. Women with PCOS show chronic anovulation, hyperandrogenism and insulin resistance. It is the main cause of infertility and it is characterized by menstrual dysfunction and metabolic disorders. Both hyperandrogenism and hyperinsulinemia play a pivotal role in the pathogenesis of PCOS. Dyslipidemia and insulin resistance, that occur in this syndrome, also cause an increase in cardiovascular risk.

Many years of studies and researches allowed us to outline a fairly exhaustive picture about the etiology of PCOS, and many steps forward have been made regarding the diagnosis of this syndrome, but there is still no certainty related to the therapy.

Certainly lifestyle management is a first - line treatment in PCOS: a proper diet, important also to obtain a correct BMI, may help in reducing insulin resistance and in restoring ovulatory cycles.

Inositol(s) – present in many foods, especially fruits and beans – plays a central role in important metabolic pathways which, in case of malfunction, is involved in the onset and development of PCOS. As proof of this, it was demonstrated that two stereoisomers belonging to inositol(s), myo-inositol (MI), and D-chiro-inositol (DCI), can improve several pathologic conditions related to PCOS. In plants, the inositol family is generally represented in the form of hexaphosphate, and phytic acid or its salts (phytates). A current diet is often poor of these substances; indeed, in the last decades the content of phytates was considerably reduced in food, because of their strong chelating effect.

Also other dietary molecules, such as omega-3 fatty acid eicosapentaenoic acid (EPA), were found useful for improving some pathologic conditions of PCOS. Currently, the dietary intake of women with PCOS is unclear and there is no research assessing dietary patterns of women with and without PCOS. The endocrine disorders may be managed by specific strategies employing sequential or combined pharmacological and non-pharmacological treatment. We know how important is the use of insulin-sensitizing compounds as putative treatments to solve the hyperinsulinemia-induced dysfunction of the ovarian response to endogenous gonadotropins. In this way, it is possible to reestablish menstrual cyclicity and ovulation, increasing the chance of a spontaneous pregnancy. Therefore, insulin - sensitizing drugs such as metformin and thiazolidines are therapeutic options, although some side effects limit their use for PCOS patients. MI and DCI act as insulin sensitizing drugs too. DCI is involved in mediating insulin activity chiefly in non - ovarian tissues, and MI displays specific effects on the ovary, mainly by the modulation of FSH - signaling and glucose metabolism. Moreover, MI may also enhance the ovarian steroidogenic function. Recent studies have shown that the best approach to PCOS therapy with inositol(s) is associating MI and DCI at the 40:1 ratio.

Our analysis aims to evaluate the available therapeutic strategies for the treatment of women with PCOS also focusing on diet in order to assess the possible impact on the clinical effects associated to it.

## INTRODUCTION

It was suggested that Polycystic Ovary Syndrome (PCOS) is an ancient disorder, arising from ancestral gene variants selected during the Paleolithic period and maintained over the past 10,000 years after the onset of Neolithic culture [1]. Hints are provided from later ancient medical records. Hippocrates noted: "But those women whose menstruation is less than three days or is meagre, are robust, with a healthy complexion and a masculine appearance; yet they are not concerned about bearing children nor do they become pregnant'' (Diseases of Women) [2]. The medieval physician Moises Maimonides (1135–1204 A.D) reported that: ''There are women whose skin is dry and hard, and whose nature resembles the nature of a man. However, if any woman's nature tends to be transformed to the nature of a man, this does not arise from medications, but is caused by heavy menstrual activity'' (Fin Liber Comm. Epidemirum VI, 8) [3].

The Italian physician Antonio Vallisneri (1661-1730) made the first accurate description of the habitus in a PCOS woman and the morphological features of the polycystic ovaries. He wrote the following observation on a "Young married peasant women, moderately obese and infertile, with two larger than normal ovaries, bumpy and shiny, whitish, just like pigeon eggs" [4]. Over a period of more than two millennia, reports describe a combination of signs, including menstrual irregularity, masculine habitus, sub-infertility, and possible obesity, which were suggestive of PCOS, already at that time.

## PCOS: DEFINITION AND DIAGNOSIS

PCOS is one of the most common female endocrine disorders with a variety of metabolic and endocrine abnormalities and clinical symptoms. It is a chronic condition with implications for morbidities, both in short-term, like subfertility and pregnancy-related complication, and long-term risks, like diabetes, cardiovascular diseases, poor quality of life and overall mortality. This complex syndrome involves the hypothalamus, the pituitary gland, the ovaries, the adrenal gland and the peripheral adipose tissue creating an

imbalance associated with three characteristic symptoms: oligo-anovulation, hirsutism and infertility.

The prevalence of PCOS for each specific population depends on the diagnostic criteria used. It is estimated that 6-10% (in some cases, 15%) of women in reproductive age are affected by this syndrome. However, the determination of the exact prevalence is problematic, owing to the intrinsic characteristics of the syndrome: the heterogeneity of the symptoms, their variability in different age ranges, the lack of overlapping biochemical parameters and the shared cut-off useful in clinical practice. This syndrome is the result of a complex series of the alteration of physiological mechanisms. The symptoms and clinical signs of this disease are not always evident, as already described in 1935 by Stein and Leventhal. They identified the syndrome on the increased dimension of the ovary, the thickening of the capsule and the presence of multiple follicular cysts covered by abundant stroma. The coming of ultrasound (in particular the transvaginal probe) has revealed that a micropolycystic ovary doesn't necessarily imply a diagnosis of PCOS, since patients with normal ovaries present clinical symptoms and laboratory features of PCOS, and vice versa. In 1990, in Bethesda, as a result of the Conference of the National Institute of Health/ National Institute of Child Health and Human Development (NIH/ NICHD), anovulation and hyperandrogenism were established as criteria for PCOS diagnosis [5, 6].

Beyond anovulation and hyperandrogenism there were other clinical features in women with PCOS, such as obesity (found in 30-60% of patients) [7], insulin resistance and hyperinsulinemia (present in 50-70%) [8], and the ratio between the Luteinizing hormone and the follicle-stimulating hormone (LH/FSH) >2 or 3 (frequency of 30-50%) [9, 10, 11].

In 2003, in Rotterdam, the European Society for Human Reproduction and Embryology (ESHRE) together with the American Society for Reproductive Medicine (ASRM), drafted a consensus document that established a worldwide standard diagnostic criteria for the PCOS.

The Rotterdam consensus includes three diagnostic criteria, and states that two of the three must be present in order to make the diagnosis.

The established criteria are as follow:

- Oligo-anovulation
- Clinical or biochemical signs of hyperandrogenism
- Polycystic appearing ovaries on ultrasound, characterized by the presence of 12 or more follicles with diameter of 2 ± 9 mm in each ovary, and/or increased ovarian volume (>10 ml).

The most recent diagnostic identification was drafted by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society in 2009. Thus, a modified version of the previous criteria emerged: hyperandrogenism including hirsutism and/or hyperandrogenemia, ovarian dysfunction, including oligo-anovulation and/or polycystic appearing ovaries, and the exclusion of other androgen excess or related disorders. [12]. The pathologies to be excluded for PCOS diagnosis are the following: Cushing's syndrome, 21-hydroxylase deficient congenital adrenal hyperplasia, hyperprolactinemia, thyroid disorders, premature ovarian failure, and androgen- secreting neoplasm.

Ultrasonographic diagnosis of PCOS examines the ovarian features. According to the Rotterdam Consensus criteria, the ovary must have a volume > 10 $cm^3$ in order to diagnose a PCOS. To calculate the ovary volume it is necessary to measure its maximum diameter on the longitudinal, transverse and anteroposterior section. In order to assess the internal aspect of the ovary it is necessary to evaluate the number of follicles. According to the Rotterdam Consensus criteria polycystic ovary should contain 12 or more follicles of 2-9 mm in diameter. This is a useful parameter to distinguish PCOS from multi follicular ovary, a transitory condition generally associated to hyperprolactinemia, hypothalamic anovulation, amenorrhea related to weight [6]. Sonographically the multifollicular ovary is morphologically characterized by a number of follicles, lower than in PCOS, distributed throughout the ovary and by the absence of stroma hypertrophy. In patients with PCOS there is an increase of echodensity of the ovary, though this is a subjective assessment that may vary depending upon the setting of the ultrasound machine and patient's body habitus. Stromal echogenicity has been described in a semi-quantitative manner; score 1 for a normal aspect, score 2 for a moderately increased aspect and score 3 for substantial increased aspect [13].

Increased stromal echogenicity is due to a combination of increased stromal volume and reduced echogenicity of the multiple follicles.

The primary defect in PCOS appears to be an exaggerated androgen synthesis and secretion, particularly by the ovarian theca cells; insulin resistance and obesity may act as triggers of this primary defect, explaining the frequent association of PCOS with obesity and insulin resistance [14, 15].

One of the mechanisms that seems to be the basis of hyperandrogenism in PCOS is the hypersecretion of LH, accompanied by normal or reduced levels of FSH. The LH, that in physiological conditions is the main regulator of androgen production by the ovarian theca, tends to be produced in larger amounts. In patients with PCOS it is possible to detect a LH/FSH ratio greater

than 2.5, due to the increase in LH production with normal or reduced levels of FSH [16]. It is present in many (but not in all) PCOS women; therefore, a normal value does not exclude the diagnosis of this disorder.

Studies on ovarian theca hormone production show that patients with PCOS have an increase in ovarian production of progesterone and androgens.

The detectable hyperandrogenism in PCOS is not only determined by a defect in ovarian steroidogenesis, but also involves the adrenal gland and it is Adrenocorticotropic hormone (ACTH) dependent. The androgen response to LH and ACTH seems to be modulated by internal autocrine and paracrine tissue mechanisms. The insulin, insulin-like growth factors (IGFs) and inhibin are counted among the many growth factors capable of increasing the cellular response to the action of these two hormones [17].

The diagnosis of hyperandrogenism is performed through laboratory investigations, by looking for increased androgen serum levels or through clinical examination, by looking for signs of hyperandrogenism, like hirsutism, even in the presence of normal levels of androgens in the blood. The evaluation of biochemical parameters will show high levels of all androgens, from the most powerful, the testosterone T, Dihydrotestosterone (DHT), 5-Androstenediol (D5-A), to the weakest like 4-Androstenediol (D4-A), Dehydroepiandrosterone (DHEA), and Dehydroepiandrosterone sulfate (DHEA-S). The levels of Estrone (E1) are increased and those of Estradiol (E2) are normal or reduced by a decreased follicular production with an inversion of the E2/E1ratio. The SHBG levels are reduced.

Clinically hyperandrogenism will manifest with seborrhea, and concomitant development of acne, hypertrichosis, or hirsutism. In most important cases these clinical symptoms are extremely evident and are accompanied by the presence of alopecia, increased muscle mass, clitoromegaly and changes in the voice tone.

Another very important feature of PCOS is represented by insulin-resistance and hyperinsulinemia. The insulin resistance is attributable to a reduced functionality of the insulin receptor due to hyper-phosphorylation of serine residues of the same receptor by a serine/threonine kinase. This hyper-phosphorylation diminishes ligand affinity, which ends up causing an attenuation of the endocrine hormone signal and the onset of the insulin-resistance condition [18]. Physiologically, insulin exerts several effects on insulin-sensitive tissues (liver, skeletal muscles, adipose tissue), favoring glucose intake, glycogen synthesis and inhibiting lipolysis. Insulin resistance appears when insulin sensitivity is impaired so that insulin can no longer exert its metabolic effects on insulin-sensitive tissue. When insulin resistance

interferes with glucose uptake in target tissues, insulin secretion is increased; the result is a compensatory hyperinsulinemia. The excessive pancreas insulin production is a serious risk factor in the onset of non–insulin dependent diabetes. Insulin resistance seems to be an aggravating factor for hyperandrogenism. This may occur through an indirect o direct stimulation mechanism.

In the first case the action on androgen synthesis would be due to the active role exerted by insulin, since it amplifies the stimulated androgen production by LH at theca cell level. This would explain the prevalence of hyperandrogenic symptoms in obese subjects with PCOS [19]. A second indirect mechanism which alters the normal values of androgens depends on the reduction of plasmatic sex hormone-binding globulin (SHBG). It was demonstrated that the hepatic synthesis of SHBG is inhibited by high glucose concentrations; therefore, a consequent increase of biologically circulating free-active androgens occurs [20].

The direct mechanism of hyperandrogenism is due to the role that insulin plays in the ovary. In fact, it has a direct effect on steroidogenesis in the granulosa cells.

Insulin, both in normal and in the polycystic ovary, works as a real gonadotropic hormone: it is an important factor in the regulation of ovarian androgen metabolism and synthesis. Particularly in obese women, the hyperinsulinemic status is related to an amplification of the steroidogenic signal with an increase of SHBG, and also total and free testosterone blood levels. The action on steroidogenesis is due to insulin binding to two receptors: a specific receptor present in the ovary and IGF-I receptor.

The probable existence of a defect in insulin signal transduction has focused attention on the role of the second messengers of insulin signals, the inositol phosphor glycans. At this point we have to briefly introduce the inositol(s) family, a topic that will be carefully analyzed hereafter in this chapter. Inositol is a hexahydroxycyclohexane, a 6-carbon ring compound with a hydroxyl group attached to each carbon of the ring. There are nine possible stereoisomeric forms of inositol, related to the epimerization of the six hydroxyl groups. Among these isomeric forms, myo-inositol (MI), the mostly represented isoform, stands out for its important biological roles. Some studies have shown that a shortage of MI can determine both a failure in response to insulin and a reduction in ovarian response to FSH [21, 22].

Another major clinical manifestation of PCOS is the menstrual cycle alteration. Anovulation and oligomenorrhea are common in women with PCOS. Morphologically, in PCOS, the ovary, when altered in its structure,

presents a large number of subcapsular antral follicles (5-8 mm in diameter). This aspect seems due to an over-recruitment of primordial follicles in the growth phase, giving rise to an increase of follicle turnover which would explain the appearance of PCO. In PCOS some factors subsequently contribute to the failure in dominant follicle selection and growth. Many hypotheses have been proposed as the primary cause of ovulatory dysfunction in these patients. The common finding of androgen overproduction by theca cells has prompted to identify the main explanation of ovulatory dysfunction in this overproduction; however, such conclusion was amply debated given that also the PCOS theca cells from ovulatory women exhibit an excess of androgen production. Another important aspect is the raised serum LH concentration found in PCOS women. The serum level of progesterone is an indicator of LH-mediated luteinization. We know that, whereas progesterone production is not evident in granulosa cells from normal ovary until the follicle has reached 10 mm in size, cells of follicles as small as 4 mm from PCOS women release progesterone, suggesting a premature luteinization [23] that could inhibit follicle growth.

Also hyperinsulinemia plays a role in this process of premature luteinization. Effectively, insulin has been shown to sensitize the follicle to the effect of LH such that the granulosa cells acquire LH receptors early, and, therefore, undergo terminal differentiation and early growth cessation.

## MANAGEMENT AND THERAPY OF PCOS

An uniform treatment for PCOS patients does not exist. Clinicians should perform an accurate evaluation of patients' characteristics, identifying the phenotypic target and, subsequently, the best-tailored treatment to manage one or more clinical issues. Lifestyle intervention should always be the first recommended approach.

Diet therapy is one of the most important and effective management strategies in PCOS. The health benefit of weight loss in overweight and obese women with this syndrome is well established. Weight reduction leads to improve insulin sensitivity and lipid profile; it reduces hyperandrogenism and regulates menstrual cycle [24, 25, 26]. We know that hyperinsulinemia encourages fatty acids to be deposited as body fat, and also it inhibits the release of fat from its depots. Body mass excess is evident in almost two-thirds of women with PCOS and is the cause of several aspects of PCOS, like hyperandrogenism, hirsutism, infertility. Another feature of these women,

linked to hyperinsulinemia, is reactive hypoglycemia following ingestion of refined carbohydrates. The importance of diet is that insulin resistance responds very well to weight reduction. An optimal diet must have appropriate calories to support the specific needs of a PCOS woman, depending on her necessity to lose weight or not. Lowering of dietary sugar load and choice of complex dietary carbohydrates reduces the postprandial excursion in serum glucose and decreases the excess of insulin secretion in response to dietary load. Low glycemic index carbohydrates are preferable, such as peaches, old-fashioned oatmeal, bran cereal, lentils, sweet potatoes and milk, but also foods with high fiber content and low glycemic index; they promote an increased level of satiety, help to control appetite, decrease hunger and are useful in weight control. Vegetables are high in fiber, minerals, vitamins and antioxidants. Non-starchy vegetables are low in calories and in carbohydrates; PCOS women can consume them in unlimited amounts, whereas starchy vegetables have a higher carbohydrate content and a bigger impact on insulin levels. It is known that eating low-glycemic index foods can decrease insulin resistance, improve androgen profile and increase plasma HDL in PCOS women [27]. In addition to these recommendations, a diet low in saturated fat and sodium, higher in fiber from whole grain, fruits, and vegetables can improve short and long term symptoms of PCOS, and decrease the risk of chronic diseases associated with insulin resistance.

In PCOS woman the frequency and regularity of eating patterns leads to higher post-prandial energy expenditure, lower energy intake and improved impaired insulin sensitivity compared to irregular eating [28]. An appropriate diet for these patients should be made of: 50% of total calories from carbohydrates with low glycemic index, 30% from fat, 20% from proteins and high in fiber.

Also physical activity is an essential key to managing PCOS. It can reduce insulin levels, improve insulin sensitivity and optimize lipid profile; furthermore, it is essential for the woman's physical and mental well-being. Exercise improves ameliorates glucose homeostasis related to an up-regulation of the expression of proteins involved in insulin signal transduction in the skeletal muscle; moreover, it significantly reduces triglycerides and increases plasma HDL [29, 30]. These changes result in a reduction of cardiovascular risk in PCOS population. For this reason physical activity should be recommended in such patients.

Concerning the diet, inositol(s) plays a central role in the functioning of important metabolic pathways which are involved in the onset and development of PCOS.

MI is the predominant isomeric form of inositol(s) that we can find in nature and in our food. MI was classified as a component of B complex and it is synthesized by human body from glucose. Liver is the key organ for its endogenous synthesis as well as kidneys. It is important for the smooth running of a wide range of cell functions, including cell growth and survival, the development and function of the peripheral nerves, osteogenesis and reproduction [31]. It acts as a second messenger, regulating the activities of several hormones such as follicle stimulating hormone, thyroid stimulating hormone and insulin. It is well known that reduced glucose tolerance, resulting from a defect in the insulin-signaling pathway, appears to be implicated in the pathogenesis of insulin resistance and the metabolic syndrome that affects many PCOS patients. We have already seen how insulin may have an important role in the pathogenesis of PCOS, both indirectly and directly.

MI and its stereoisomer, D-chiro-inositol (DCI), seem to play an important role as second messengers of insulin. They are capable of exerting an insulin-sensitizing effect leading to a reduction in insulin levels in the blood, whereas, they have different roles as mediators of insulin, which lead to different functions within the cells [32].

Although in many tissues, almost >99% of the intracellular inositol pool is constituted by MI, significant differences have been observed in MI and DCI concentration in fat, muscles and liver. This different distribution is due to the different roles that these two stereoisomers have within the tissues. A direct enzymatic transformation of MI to DCI maintains this proportion within the cell [33, 34, 35]. Larner showed that each organ has a specific MI/DCI ratio [36].

MI plays a central role in morphogenesis, cytoskeleton rearrangement, glucose metabolism, regulation of cell proliferation and fertility. In the latter case, MI regulates gamete development, oocyte maturation, fertilization and early embryonic development. It has a role as a second messenger of calcium signaling in oocyte growth, during zygote development, another process mediated by the modification of intracellular $Ca2^{+}$ oscillation [37, 38]. As it is now clear, the importance of fluctuations in $Ca2^{+}$ levels during the process of oocyte maturation, fertilization and embryogenesis is linked to bioavailable MI. The presence of high concentration of MI in the follicular fluid has become a marker of good quality oocytes [37-42]. Through these mechanisms, MI leads to a restoration of spontaneous ovulatory cycles in patients with PCOS.

Human diet from animal and plant sources can contain MI in its free form, as inositol-containing phospholipid (phosphoinositides) or as phytic acid

(inositol hexaphosphate or IP6). The greatest amounts of MI in common foods are found in fresh fruits and vegetables, and in all foods containing seeds (beans, grains and nuts). Especially high phytic acid contents are found in almonds, walnuts and Brazil nuts [43] and oats and bran contain more MI than cereals derived from other grains. Among vegetables, the highest contents are found in the beans and peas, leafy vegetables are the poorest vegetable sources. Among the fruits, melon and citrus fruits (with the exception of lemons) have extraordinarily high contents of MI. But although it is contained in various foods the current diet is poor of these substances. Indeed, in the last decades the content of phytates was considerably reduced in food since their strong chelating effect. Phytic acid forms strong complexes (phytates) with many essential bi- and trivalent metal ions in foods as well as in the intestine. The presence of phytic acid may, therefore, have an effect on the bioavailability of minerals. Due to the chelating properties of the different dietary fiber components and the phytic acid, there has been a lot of concern about the effect of an unrefined high-fiber diet on mineral availability. Indeed, in earlier studies it was claimed that phytic acid was the component mainly responsible for the chelating of divalent minerals. More recently it has been suggested that phytic acid is not the component that is solely responsible for the decreased mineral availability, as dietary fiber itself also might be of importance [44, 45].

As most plant foods, such as whole grain products - the main sources of dietary phytate intake – are processed or heat treated either during food production or preparation, phytases in several processed foods are inactivated or removed to a large extent [43, 46]. For this reason the amount of MI absorbed with the diet nowadays is reduced.

Another dietary molecule such as omega-3 was found useful for improving some pathologic conditions of PCOS. The obesity and abdominal adiposity, androgen excess, and insulin resistance can develop oxidative stress, [47] generating a condition with significant decrease in serum antioxidant and vitamins levels and these women are in an increased risk of oxidative status [48]. Oxidative stress and antioxidant decrease may increased risk of cardiovascular disease, insulin resistance, hypertension, central obesity, and dyslipidemia in patients with PCOS. [49, 50]. Fish oil is the main source of dietary omega-3 fatty acids. They have several healthy effects including anti-inflammatory, antithrombotic, antiarrhythmic and antiatherogenic effects. The ω-3 also may be effective in decreasing hirsutism, BMI, LH, testosterone, insulin and increasing sex hormone-binding globulin (SHBG) in women with PCOS [51]. Supplementation with omega 3 would seem to promote the

regularization of menstrual cycles. This result can be due to effects of ω-3 on testosterone concentration [52]. Furthermore, these molecules are useful for PCOS women in order to reduce lipids and lipid peroxidation levels [53].

The endocrine disorders present in PCOS could benefit by specific strategies employing sequential or combined pharmacological and non-pharmacological treatment. COCs are the first-line approach in PCOS women with no reproductive desire and with moderate or severe hirsutism and menstrual abnormalities.

The efficacy is due to hormonal action at central and peripheral level. Progestins and estrogens suppress the luteinizing hormone release and subsequently decrease ovarian androgen production. The estrogens increase the liver production of sex hormone binding protein (SHBG), which decreases the plasmatic level of free androgens.

Low-dose of combined oral contraceptives containing EE2 and antiandrogenic progestins, are the most employed drugs in clinical practice. The best EP compound to treat hyperandrogenism has not been identified but there is a wide consensus on avoiding progestin with high androgenic power [54-57]. The levonorgestrel has been recently recommended as one of the progestagens with less thrombogenicity and unprovided of androgenic effects [58]. Antiandrogenic progestins act through different mechanisms: antagonizing androgen receptor - cyproterone acetate, drospirenone, dienogest- or inhibiting 5a-reductase activity - cyproterone acetate, chlormadinone acetate, desogestrel, gestodene, norgestimate, drospirenone, dienogest.

Drospirenone is a progestogen derived from 17a-spirolactone. Unlike other progestins, it has anti-mineralocorticoid and antiandrogenic properties and is pharmacologically similar to endogenous progesterone. It blocks the action of testosterone binding to androgen receptors and this can reduce androgen effects on facial hair, lipids and insulin [59]. It seems more effective on acne and seborrhea than other estroprogestinics [60].

Another molecule is cyproterone acetate. It is the oldest progestin used for its antiandrogenic properties, commonly associated with estradiol. Compared with placebo, cyproterone acetate induces a significant improvement in hirsutism, but compared with other estroprogestinics no significant differences were observed [61, 62].

Estradiol valerate is a new molecule under study. It is similar to and has a shorter half-life than 17b estradiol, thus, it might have less impact on lipid and glucose metabolism and a decreased risk for thromboembolic or CV complications. A small preliminary observational study suggested that, after 1-

year of treatment, the estradiol valerate, associated with a progestin, exerts a positive influence on acne and hyperandrogenism in PCOS women with mild or moderate acne [73].

After 6 months of COCs or after COCs failure the antiandrogens are the most effective drugs currently available for the hirsutism management. All antiandrogens have a teratogenic effect, due to the block of androgen receptors that induces a feminization of male foetuses. Thus, during treatment period, patients should use adequate contraception method.

International guidelines recommend that after 6 months of EP treatment without hirsutism improvement, a combined therapy of EP plus antiandrogens should be performed [64].

About menstrual cycle alterations, progestins are administered to treat menstrual disorders in oligomenorrhoic PCOS women. Cyclical progestogens are employed during the second half of the menstrual cycle, in order to get a regular withdrawal bleed. The continuous progestogens are used to induce endometrial atrophy and hence to prevent estrogen-stimulated endometrial proliferation. This type of therapy, however, causes a number of side effects such as CV complications, depression and hydric retention. However, the literature data indicate the combined oral contraceptives as a first-line pharmacological strategy for menstrual disorders in PCOS women without reproductive desire [54]. Considering the phenotypic characteristics of the patients, usually a low-dose of EP, with antiandrogenic progestins, is prescribed. The treatment changes in presence of women who want pregnancy. Ovulatory cycles are aimed to be restored in order to ensure a greater chance of pregnancy. Usually, these patients are treated with clomiphene citrate, which restores ovulation by blocking estrogen receptor at the hypothalamic level, reducing the negative feedback of estrogens and increasing endogenous FSH-LH secretion [65]. If ovulation cannot be induced with this therapy, the patient is considered to be clomiphene resistant, and an alternative treatment has to be scheduled.

Another therapeutic approach aimed to establish ovulatory cycles involves the administration of Insulin-Sensitizing Drugs.

Between these molecules the most used is the metformin. It is a biguanide approved for the management of type 2 diabetes mellitus. It appears to act by inhibiting hepatic production of glucose, reducing oxidation of fatty acids and increasing peripheral tissue uptake of glucose [66]. Treatment with metformin leads to a marked improvement of hyperinsulinemia, reduction of hyperandrogenemia, restoration of ovulatory function, increase of pregnancy rates and reduction in first-trimester spontaneous miscarriage [67]. However,

metformin effects do not seem to be the same in all PCOS women. Whereas, metformin administration significantly reduces levels of fasting insulin in non-obese women with PCOS, it fails to show a similar effect in the obese PCOS women. Keeping in mind the considerable side effects of metformin, this drug may not be recommended for fertility management in these women. However, it would appear that a beneficial effect can be achieved in these patients, with the administration of metformin in association with clomiphene citrate [68]. We must also remind that, though metformin has no fetotoxic effects in humans, side effects (bloating, abdominal discomfort, nausea vomiting and diarrhea) may occur restricting its use.

## PCOS: MYO-INOSITOL AND D-CHIRO-INOSITOL

Studies carried out on PCOS and the therapeutic benefits derived from the administration of substances reducing insulin resistance, have prompted to employ MI and DCI in the treatment of patients affected by this syndrome. The rationale underlying the therapeutic application of inositol(s) in PCOS derives from their activities as insulin sensitizing agents and their healthy effects on metabolism. As briefly outlined before, both MI and DCI function as insulin second messengers and mediate different insulin actions. MI is converted to an inositolphosphoglycan (IPG) insulin second messenger (MI-IPG) involved in cellular glucose uptake, whereas DCI is converted to an IPG insulin second messenger (DCI-IPG) involved in glycogen synthesis [69]. We have to add that, at ovarian level, MI based second messenger is involved in both glucose uptake and FSH signaling whereas DCI-based second messenger is devoted to the insulin-mediated androgen production. Several studies have shown that both DCI and MI are able to reduce LH levels, LH/FSH ratio and testosterone levels, to restore spontaneous ovulation and menstrual cycles [70] and to improve cutaneous disorders of hyperandrogenism, reducing hirsutism and acne [71].

John Nestler and his team, provided evidence that the impairment in insulin signaling in PCOS could be the result of a defect in the IPG insulin second messenger pathway, consistent with the insulinomimetic role of IPGs in activating enzymes which control glucose metabolism. In PCOS women, a deficiency of IPGs in tissues, or altered metabolism of inositol(s) to IPG mediators, could play a role in inducing insulin resistance [72]. We have seen that increasing insulin sensitivity in PCOS patients by means of conventional antidiabetic drugs results in an improvement of ovarian function and the

decrease of serum androgen concentrations. Moreover, it was demonstrated that metformin increases the release of insulin-stimulated DCI phosphoglycans, thus evidencing that this antidiabetic drug may enhance insulin sensitivity by restoring the INS-based signal. It was further observed that PCOS patients suffer from an altered DCI urinary clearance, presumably leading to tissue depletion of IPG [73]. Once more, these data suggest a direct correlation between the availability of IPG and insulin resistance. This condition is associated with abnormally low levels of DCI and excessive levels of MI in urine with intracellular MI deficiency in insulin-sensitive tissue.

Starting from these results, many clinical trials have been performed in order to evaluate the clinical usefulness of DCI supplementation in PCOS treatment.

Nestler et al. [70] demonstrated that in obese women with PCOS, DCI treatment at a dose of 1200 mg/day reduced serum testosterone level and improved ovulation rate and metabolic parameters such as blood pressure and triglycerides. In over 70% of patients, previously in amenorrhea, the menstrual cycle occurred within 45 days of treatment. Further studies were carried out involving a larger number of patients and with increasing doses of DCI, up to 2400 mg/day [72]. Unfortunately, and unexpectedly, the authors in this study were not able to confirm the results published previously. A crucial difference from previous studies was the dose of DCI administered. The PCOS patients treated with increasing DCI doses (from 300 to 2400 mg/day) provided a compelling confirmation that such augmentation progressively worsened both oocyte quality and ovarian response. The contradictory results could likely be explained by considering the different function that each inositol stereoisomer plays in distinct tissues. Indeed, a specific MI/DCI ratio has been observed in different tissues: high DCI levels (even if always lower than MI concentration) are generally observed in glycogen storing tissues (fat, liver, muscle), whereas low DCI levels are present in tissues characterized by high consumption of glucose (brain, heart). MI seems to play a more important role in oocyte: in fact, almost 99% of intracellular INS pool is constituted by MI [74]. DCI is produced from MI through a nicotinamide adenine dinucleotide-dependent epimerase according to cells requirement. Indeed, the epimerase conversion of MI to DCI is under insulin control. In fact in Type 2 diabetic patients, the reduced tissue insulin sensitivity leads to reduced epimerase activity and hence DCI synthesis. However, unlike other tissues, the ovary never shows insulin resistance. Unfer et al. suggested that in women with PCOS, hyperinsulinemia likely overstimulates epimerase activity in the ovary, resulting in an excessive production of DCI and a concomitant depletion of MI. In particular, the

MI/DCI ratio decreased from 100:1 in healthy women to 0.2:1 in PCOS women [22]. Furthermore, these data clarify the clinical evidence on the link between MI and FSH and on the inconsistent data present on DCI.

The authors postulated that the resulting deficiency of MI could be responsible for the poor oocyte quality and the impairment of the FSH signaling. Clearly, DCI supplementation would be ineffective in such women as they already have high levels of this molecule in the ovary [22, 72]. Certainly, the administration of high doses of DCI determines ovarian toxicity, which progressively reduces ovarian response to FSH and adversely affects oocyte quality. This could explain why the promising results obtained by Nestler and co-workers during the first study have not been confirmed in the second one, when he has doubled the dose of DCI given to patients.

Thus, while DCI increase may promote androgen synthesis, MI depletion worsens the energy state of the oocytes. These events, together, impair FSH signaling and oocyte quality. This imbalance could clarify the pathogenesis of PCOS and better explain the theory called "the DCI paradox in the ovary", first suggested by Dr. Unfer and co-workers [73].

The insulin boost causes an enhanced action of epimerase in the ovary, resulting in excessive conversion of MI to DCI. Therefore, in PCOS, there are follicles in which DCI is abundant, while MI is present at low levels [74], causing a lack of second messengers. The impairment of transmission of this signal could be responsible for chronic anovulation that occurs in PCOS. Several lines of evidence support this hypothesis. Studies performed on women undergoing assisted reproductive technologies have shown that women with increased fasting insulin levels required more international units of rFSH in order to achieve proper ovarian stimulation [75]. Furthermore, MI administration to PCOS women undergoing IVF was associated with a reduction in the quantity of rFSH administered and the number of days of stimulation [76]. These evidences demonstrate that MI improves FSH sensitivity, providing further support to the idea that MI administration beneficially affects ovarian function and oocyte development.

At this point, it was important to understand the physiological concentrations of MI and DCI in plasma in normal women to identify the presumably optimal clinical dosage [77, 78]. The ratio of MI to DCI in plasma was found to be approximately 40:1. Based on this information, a treatment was established using a soft gel capsule containing 550 mg of MI and 13.8 mg of DCI that reproduced the MI/DCI physiological ratio in plasma [77, 79]. This hypothesis was supported by the results obtained in three clinical studies. In the first study, the combined MI plus DCI therapy (40:1), versus MI alone,

was able to restore hormonal and metabolic parameters in overweight PCOS women earlier than using only MI. Indeed, plasma glucose and insulin concentrations showed a significant reduction in the study group. Also serum sex hormone levels improved. In particular, compared to the MI group, the decrement of total testosterone and the increment of the serum sex hormone binding globulin were more relevant in MI plus DCI patients [80]. In another study, in obese PCOS women, the combined therapy MI plus DCI improved the metabolic profile (LDL, HDL, triglycerides), reducing the cardiovascular risk. Furthermore, the Homeostasis Model Assessment (HOMA) index significantly got better [81]. Finally, also the results in oocyte quality obtained with this therapy resulted interesting in PCOS women undergoing IVF-ET. Significant better results were observed in the MI-DCI (40:1) group when compared to controls receiving DCI 500 mg. Patients treated with MI-DCI (40:1) required lower dosages of rFSH for a shorter period of time, and showed a significant improvement in both oocyte quality and pregnancy rate [82] Furthermore, the recent Inositol Consensus Conference on the use of MI and DCI in obstetrics, gynecology and fertility [83] formally affirmed the framework suggested by Unfer, adding a new milestone to the promising story of inositol(s).

The US FDA declaring inositol(s) generally recognized as a safe (GRAS) molecule, but the main data, on which FDA has based its decision, refers to MI. Indeed, several studies have been carried out using MI in a dosage up to 30 g/day. In these studies, the only side effects identified were reported as gastrointestinal discomfort [72]. Therefore, it has not only several beneficial effects, but it is also a safe compound to be administered during pregnancy [84].

Conversely, no data are available for DCI. Indeed, from a careful analysis of the available literature, it can be inferred that DCI administration at relatively high dosage has detrimental effects at ovarian levels.

## CONCLUSION

To conclude, the treatment for PCOS depends on the patient's phenotype, the symptomatology, and the desire, or not, of pregnancy. Changes in lifestyle such as increased physical activity, healthy diet and body weight reduction are fundamental. Patients who do not wish to get pregnant in early age, can control hyperandrogenism symptoms and menstrual irregularities by taking

oral contraceptives, which can also be associated with oral anti-diabetic drugs to obtain a better control of insulin resistance.

Instead, ovulation can be restored in women who wish to get pregnant by clomiphene citrate administration. Whereas, in patients not responding to this stimulation, assisted reproductive technology of second level should be employed.

The latest findings regarding the likely pathogenesis of PCOS prompt us to include also inositol(s) in the first-line treatment choices.

All the studies performed in PCOS and, mainly, those ones showing the usefulness of both stereoisomers, led to assert that a synergistic action of these chemically, but not functionally, "twin" molecules is certain. Therefore, their association in the therapy for PCOS is highly recommended. It was important to detect the altered intracellular relationship between MI and DCI in PCOS patients and identify the right ratio between them; it allowed finding a therapeutic approach for the treatment of PCOS without incurring in ovarian toxicity related to the amount of DCI administered.

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